The Genetic Structure and Dispersal Patterns of the Nigeria-Cameroon Chimpanzee (*Pan troglodytes ellioti)*

A thesis submitted in partial fulfilment of the requirements for the Degree of

Master of Science in Evolutionary Ecology

Department of Biological Sciences

University of Canterbury

By Alexander Knight

2013

Table of Contents

Chapter 1 Introduction

Chapter 2 Methods

Chapter 3 Genetic Structure and Inbreeding

Chapter 4 Sex Biased Dispersal

Chapter 5 Population Size and Viability Analysis

Chapter 6 Conclusions and Conservation Strategy

List of Figures

List of Tables

Acknowledgments

So many people have contributed in different ways to making this thesis a reality and I am grateful to everyone for their assistance. I particularly owe thanks to my supervisors, Dr Hazel Chapman and Dr Marie Hale, for their continuous guidance and wisdom throughout the course of the project, Maggie Tisch, for her assistance in everything laboratory related and more, my collaborators, in particular Dr Katy Gonder for her encouragement and technical assistance, Dr Bethan Morgan for taking the time to support my grant applications and Dr John Oates for his advice and assistance. I would like to thank Conservation International, the Mohammed bin Zayed Species Conservation Fund and the Montane Forest Conservation Initiative (formerly the Nigerian Montane Forest Project) who provided financial support that made this thesis possible, all my colleagues on the fifth floor of the laboratories at the University of Canterbury who made the lab such a fun place to work, all my colleagues in Nigeria who spent five arduous months trekking round the Nigerian bush looking for chimpanzees with me and finally my parents for making my university career possible.

Chapter 1 Introduction

The taxonomy of chimpanzees

Two species of chimpanzee are currently recognised, the common chimpanzee, *Pan troglodytes* and the pygmy chimpanzee or bonobo, *Pan paniscus* (Morin *et al.* 1994, Groves 2001). The taxonomy of chimpanzees has been a contentious issue ever since their acquaintance with European explorers over 200 years ago. The nomenclature 'troglodytes' (Latin for 'dweller of caves') was first assigned to chimpanzees by the German naturalist Johann Blumenbach in 1775 (Groves 2005) who named a specimen *Simia troglodytes*. Between then and the early twentieth century over 25 scientific names were attributed to different specimens (Groves 1986). Coolidge (1933, cited by Groves 2005) is credited with separating the bonobos and common chimpanzees into two species. It was Schwarz (1934, cited by Groves 2005) who first characterized the modern taxonomic grouping which divided the common chimpanzee into three sub-species.

Chimpanzees are distributed across Africa, from Senegal in the west to Tanzania in the east (see Figure 1.1). The common names for the three sub-species that Schwarz (1934, cited by Groves 2005) described are derived from their respective locations in Africa. Western chimpanzees (*P. t. verus*) were considered to exist in two large populations, the first occupying forests between Senegal and Benin. The second population inhabited Nigeria, as far east as the Niger River which separated them from central chimpanzees (*P. t. troglodytes*). The range of central chimpanzees was believed to stretch from the Niger River across central Africa to the Ubangi River in the Democratic Republic of Congo and as far south as Cameroon. The third sub-species, eastern chimpanzees (*P. t. schweinfurthii*) exist east of the Ubangi River all the way to Tanzania (Gonder *et al.* 2006).

This long held view of chimpanzee systematics was thrown into disrepute when Gonder *et al.* (1997) sequenced the mitochondrial DNA (mtDNA) of 12 chimpanzee hair samples. The samples were collected near the Niger River, the putative divide between western and central chimpanzees. Gonder *et al.* (1997) argued that mtDNA sequences from these new Nigerian samples were sufficiently different to warrant the classification of a new sub-species. Gonder *et al.* (1997) indicated that the samples were more similar to western

than to central chimpanzees, which some of them would have previously been classified as under Schwarz's taxonomic system. Gonder *et al.* (1997) proposed that either a new sub-species be classified, *P. t. vellerosus* (later becoming *P. t. ellioti*, Oates *et al.* 2009) or central and eastern chimpanzees be incorporated into one sub-species; reasoning that the Nigerian samples differ from western chimpanzees at least as much as central and eastern chimpanzees differ from each other.

Since then, a phylogeographic debate on the number of sub-species that exist has ensued. Stimulated by Gonder's findings, Groves (2001) examined skeletal specimens of chimpanzees. Craniometric measurements of specimens sourced from either side of the Niger River, within the range of Gonder's newly proposed sub-species, were similar to one another. Contrary to Gonder's genetic data, however, these specimens showed more resemblance to eastern and central chimpanzees than western.

Gonder *et al.* (2006) strengthened their previous findings by compiling a data set comprising of 254 mitochondrial haplotypes which included 79 unique haplotypes from the vicinities of the Niger and Sanaga rivers. This more comprehensive study supported their hypothesis, grouping western and Nigeria-Cameroon chimpanzees into one monophyletic clade, and central and eastern chimpanzees into another, the location of the biogeographic divide for the two clades being found at the Sanaga River in Cameroon. A subsequent analysis utilizing microsatellite data, however, provided only weak evidence of a geographic divide at the Sanaga River but enervated the role of the Niger River as a barrier to gene flow (Gonder and Distotell 2006). A study by Becquet *et al.* (2007) showed similar results. Cluster analysis of 84 individuals based on 310 microsatellite loci supported the taxonomy of three sub-species; western, central and eastern. Only tentative support for a fourth sub-species was evidenced with a principal components analysis. A significant differentiation between the Nigeria-Cameroon chimpanzees and western chimpanzees was shown when data on these two populations were considered alone, but not when all sub-species were analysed. Becquet *et al.* (2007) reported issues with the known origins of chimpanzee samples used in the study and the relatively small sample size, both of which likely hindered validation of a fourth sub-species, but concluded that the study indicated its existence.

With the exception of Groves' (2001) morphological data there has been little other forms of evidence to distinguish the new sub-species. This is probably due the substantial investment required in chimpanzee research, especially behavioural studies. Long term field sites have been established in the ranges of western, central and eastern chimpanzees (Mitani *et al.* 2002). Long term scientific observations on the Nigeria-Cameroon chimpanzee have begun only relatively recently (Sommer *et al.* 2004). Furthermore variation in

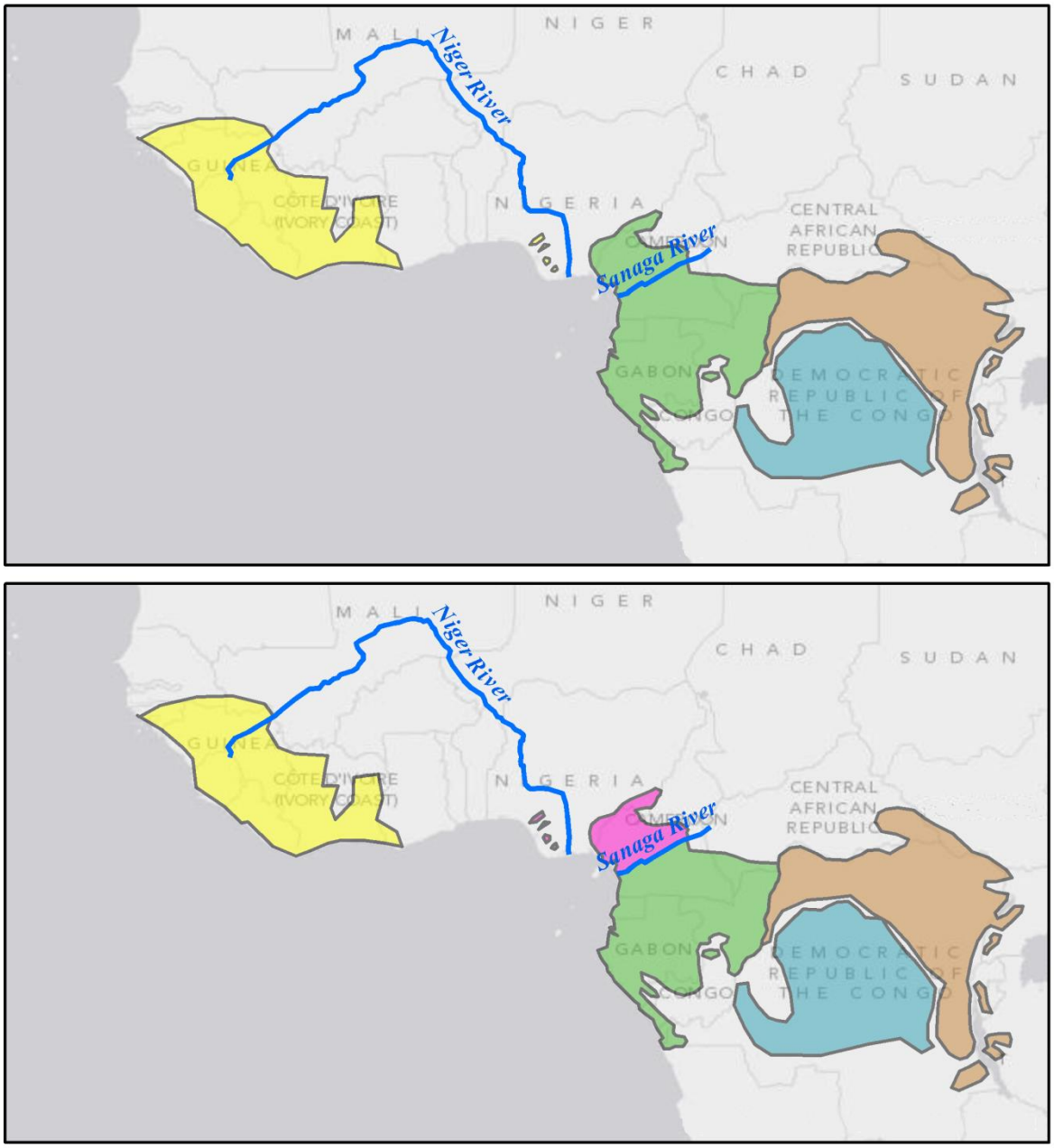

Figure 1.1: Top: distribution of the bonobo (*Pan paniscus*) and the three sub-species of common chimpanzee (*Pan troglodytes spp.*) before the identification of the Nigeria-Cameroon chimpanzee. Below: the current recognised distribution of the four common sub-species of chimpanzee and the bonobo.

chimpanzee behaviour may be cultural rather than genetic and present at a scale much smaller than that proposed for the delimitation of sub-species (Whiten *et al.* 1999).

The latest analysis from Gonder *et al.* (2011) based on 27 microsatellite loci of populations from across Africa continues to support the genetic distinction of the Nigeria-

Cameroon chimpanzee. Calculation of the most recent common ancestor relates them closer to central and eastern chimpanzees than to western chimpanzees, giving credence to the morphological comparisons of Groves (2001). Despite the validity of the genetic evidence for different sub-species being questioned by some authors (Fischer *et al*. 2006), the taxonomic distinction of four sub-species is now largely accepted (Inskipp 2005, Morgan *et al.* 2011, IUCN 2013). Figure 1.1 illustrates their current distribution in relation to the other three sub-species and the bonobo.

Although there is widespread acknowledgement of *P. t. ellioti* as a distinct sub-species, there appears little respite in this vigorous debate. Gonder *et al.* (2011) suggested there is genetic evidence to consider another sub-species in the south of Cameroon, and contested the distinctiveness of eastern chimpanzees. Whereas Groves' (2005) study of morphological variation would imply eastern chimpanzees be split into two sub-species, *P. t. schweinfurthii* and *P. t. marungensis*. Furthermore there is support for the elevation of western chimpanzees to full species rank as they appear to form a monophyletic clade of early origin (Morin *et al.* 1994, Gonder *et al.* 2011).

Chimpanzee demography

The lifespan of chimpanzees observed in the wild typically lasts into their late thirties or forties, although some individuals have been known to survive and reproduce into their fifties (Goodall 1986, Hill *et al.* 2001, Emery Thompson *et al.* 2007). Their life cycle is classified into several distinct stages defined by their behaviour and physiology (Goodall 1986, Nishida 1990).

Infancy

The duration of infancy in chimpanzees lasts for the first four to five years from birth (Goodall 1986). During this time infants are dependent on their mother for food and transport. The end of this stage coincides with the transition to a completely solid food diet and a more independent means of travel (Goodall 1986). If the mother dies during this period then the infant will not survive (Nishida 1990).

Adolescence

The adolescence period is characterized by an increasing independence leading up to the age of first reproduction. Although adolescents engage in sexual activity it is not until the next stage, maturity, when reproduction occurs (Goodall 1986). As they approach maturity males become more integrated into the adult social hierarchy and females begin to develop regular sexual cycles and form sexual associations with males (Goodall 1986).

Observations on *P. t. schweinfurthii* revealed that females can mature slightly earlier than males at around the age of 13 or 14 years old whereas males mature between the ages of 13 and 15 (Goodall 1986, Nishida 1990). In *P. t. verus* the age of first reproduction has been observed in an individual as young as nine years old (Sugiyama 2004).

Maturity

During adulthood or maturity males vie for rank in social groups and reproductive opportunities while females spend much of their time raising offspring (Goodall 1986). The reproductive life of female chimpanzees is correlated with senescence patterns, fertility peaks in their mid-twenties to early thirties and declines steadily afterwards as they senesce. Unlike humans, chimpanzees have no or very short post reproductive life spans (Emery Thompson *et al.* 2007). Thus reproduction usually lasts into their forties but has been recorded as late as 55 years old in *P. t. schweinfurthii* (Emery Thompson *et al.* 2007). Male rank is related to reproductive success but young males reproduce with less 'attractive' females (Wroblewski *et al.* 2009). Females give birth approximately every four or five years although if the offspring die during infancy then sexual cycles can be resumed in a couple of months (Wallis 1997).

Old age

Chimpanzees begin to show noticeable signs of aging in their thirties (Goodall 1986). Greying hair, balding, worn teeth and a decrease in activity are all symptomatic of old age in chimpanzees.

Community structure and sociality

Chimpanzees live in multi-male multi-female groups known as communities or unit-groups (Nishida 1968, Goodall 1986). The size of communities varies substantially, from as few as twenty individuals (Sugiyama and Koman 1979) to over 150 (Mitani and Amsler 2003). Communities exist in a fission-fusion model of society that consists of one large group which periodically divides into smaller sub-groups or 'parties' that vary in the number and composition of individuals (Nishida 1968, Mitani *et al.* 2002). Research has shown that party size is influenced by the size of the community (Lehmann and Boesch 2004), food availability and the number of estrous females (Anderson *et al.* 2002).

Males are philopatric and are known to be more gregarious and sociable than females (Nishida 1990, Mitani *et al.* 2002). Associations between males are not random, coalitions and long term association form between predictable members of a group (Mitani

and Amsler 2003). Males cooperate in a number of activities including hunting, patrolling of boundaries and mate guarding (Boesch 1994, Watts 1998, Watts and Mitani 2001).

The philopatric behaviour of males has invoked the role of kin selection theory to explain the cooperative behaviour in chimpanzees (Morin *et al.* 1994). There is evidence that males form long lasting equitable bonds with maternal kin (Mitani 2009) although certain studies have found that individuals that cooperate in certain behaviours are not related (Goldberg and Wrangham 1997, Mitani *et al.* 2000, Mitani and Amsler 2003) but can be similar in age and rank (Mitani and Amsler 2003). Some have argued that bonds between males are driven by opportunistic 'political' or social motives rather than altruism between kin (de Waal 1984).

Territoriality

Intergroup conflict amongst neighbouring communities of chimpanzees compels conclusions that they occupy, defend and attempt to expand well defined territories (Wilson and Wrangham 2003, Mitani *et al.* 2010). Lethal intergroup encounters and territorial expansions seem to be in stark contrast to earlier descriptions of overt sociability and the fission-fusion nature of their communities (Mitani *et al* 2002). One particular enigmatic situation occurred at Gombe, Tanzania when a group of males violently usurped another group that had recently fissioned from the community of the attackers (Goodall 1986, Mitani *et al.* 2010). Recent studies now imply that hostile attitudes towards neighbouring communities are the norm but the nature of specific interactions depend on the demographics of the individuals involved (Wilson and Wrangham 2003, Boesch *et al.* 2008). The purpose of costly intergroup aggression appears to be the gain in food resources and mates (Wilson and Wrangham 2003, Mitani *et al.* 2010). Williams *et al.* (2004) argued territory defence was driven primarily by food resource competition. A direct consequence of defending food resources was the increase in the reproductive success of females within that territory. The cost of aggressive behaviour may well be calculated by the assessment of numerical advantages in confrontational situations (Wilson *et al.* 2001). Responses by males to experiments of simulated invaders were dependant on the number of males in the party (Wilson *et al.* 2001).

Long term studies of territory size have consequently shown that they fluctuate in response to the outcomes of territorial behaviour (Mitani *et al.* 2010). In Ngogo, Uganda, following forays of parties from a large community of approximately 150 individuals into the periphery of their range, their territory expanded by approximately six km^2 in size contributing to total territory size of roughly 34 km^2 (Mitani *et al.* 2010). In Taï Naional Park, Côte d'Ivoire, comparisons of communities have revealed slightly smaller territories ranging

between approximately nine and 26 km^2 (Herbinger *et al.* 2001). General heuristic theories of territory size predict it is related to the defensibility of the area ergo smaller population sizes are likely to be associated with smaller territories (Lowen and Dunbar 1994). Support for this hypothesis within chimpanzees has been equivocal (Willams *et al.* 2004).

The Nigeria-Cameroon chimpanzee

Since its classification as a distinct sub-species, the taxonomy and genetics of the Nigeria-Cameroon chimpanzee have been the focus of a number of studies (Groves 2001, Gonder and Disotell 2006, Gonder *et al.* 2011). There is a paucity of knowledge around other aspects of the sub-species' biology which, until recently, has been due to the lack of long term field sites. In the last decade a number of research projects such as the Ebo Forest Research Project in Cameroon and the Gashaka Gumti Primate Project and the Montane Forest Conservation Initiative in Nigeria have begun consistent field work on the sub-species. Below I have outlined some of the significant discoveries to have emerged from these projects as well as recapping the history behind the name.

Nomenclature

When the distinctiveness of the Nigeria-Cameroon chimpanzee was first recognized it was attributed the scientific name *Pan troglodytes vellerosus* (Gonder *et al.* 1997). The name was chosen because this was the first name believed to be associated to a specimen from this sub-species (Gonder *et al.* 1997, Oates *et al.* 2009). This early specimen was actually collected by a nineteenth century explorer, Richard Burton, in Gabon, part of the range of *P. t. troglodytes* (Oates *et al.* 2009). It was mistakenly believed to have come from Mount Cameroon after John Gray, Keeper of Zoology at the British Museum placed a description of the specimen amongst other species coming from Cameroon, thus making *P. t. vellerosus* a synonym of *P. t. troglodytes.* When this fact came to light the sub-species was re-named *Pan troglodytes ellioti* (Oates *et al.* 2009). This was the name Paul Matschie, Curator of the Berlin Museum named a specimen from Cameroon in the early twentieth century in honour of Daniel Giraud Elliot, an American zoologist, for his work on ape natural history (Oates *et al.* 2009).

To date the name *P. t. ellioti* has become the recognised nomenclature of the sub-species (Gonder *et al.* 2011, Morgan *et al.* 2011). Common names for the sub-species include the Nigeria-Cameroon chimpanzee (Morgan *et al* 2011), the Nigerian chimpanzee (Fowler and Sommer 2007) and the more colloquial 'fourth chimpanzee' (Fowler *et al.* 2011). In the local Hausa dialect chimpanzees are called 'Biri mai ganga' that translates to 'the monkey with the drum' a reference to their tree drumming behaviour (Nyanganji *et al.* 2011).

Community and party Size

Published results on the demography of the Nigeria-Cameroon Chimpanzees are only available from the population at Gashaka Gumti National Park (GGNP) and Ngel Nyaki forest reserve (Sommer *et al.* 2004, Beck and Chapman 2008). At Ngel Nyaki the population size was estimated at 12.5 individuals and the forest there is approximately seven km^2 (Beck and Chapman 2008). The Gashaka-Kwano community in GGNP is estimated to consist of 35 individuals with a home range of approximately 26 km^2 (Sommer *et al.* 2004). The average party size observed during the day at GGNP was 3.7 individuals (range 1 – 17) and average nest group size was 5.7 individuals (range 1 – 23, Fowler *et al.* 2011). The age composition of the community was estimated at 81% adults, 9% juveniles and 10% infants (Sommer *et al.* 2004).

Ethology

Much of the ethological work so far has reported on the tool use of the Nigeria-Cameroon chimpanzee. Intraspecific variation in behavioural traits, particularly the use of tools, can be interpreted as a form of culture (Boesch and Boesch 1990, Fowler *et al.* 2011). There is no unanimous definition of culture (Fowler *et al.* 2011) but the term is often applied to socially learnt behavioural traits (McGrew 1994). Chimpanzees show substantial variation in tool use behaviour not just among sub-species but also between populations within sub-species (Whiten *et al.* 2001). In some instances this variation can be explained by ecological restraints, such as the food sources or materials for tools not being present preventing the requirement or ability to manufacture specific tools (Boesch and Boesch 1990, Whiten *et al.* 2011). Ecological restraints do not explain all the variation observed, nor is it likely that genetic factors influence the distinctions thus culture is invoked as the source of variation (Wrangham 1994, Whiten *et al.* 2001).

Research on tool use by the Nigeria-Cameroon chimpanzee is hampered by the lack of a fully habituated community and long term observations (Sommer *et al.* 2004, Fowler and Sommer 2007). Studies thus far have relied on indirect methods of collecting and examining discarded tools (Fowler and Sommer 2007). Tools that have been recovered in GGNP are believed to be used for harvesting ants and probing bee hives (Fowler and Sommer 2007, Fowler *et al.* 2011). Termites do not appear to be harvested by the community at GGNP (Fowler *et al.* 2011), yet at Ebo Forest termite fishing sticks have been found (Abwe and Morgan 2008). The explanation for this may be ecological as termites occur at low densities in the forests of GGNP (Fowler and Sommer 2007).

Cultural variants in the behaviour of the Nigeria-Cameroon chimpanzee are possibly nut cracking and the stripping of bark from sticks (Fowler and Sommer 2007). The ecological requirements for nut cracking exist in GGNP but it is not observed there yet it has been reported in populations of the Nigeria-Cameroon chimpanzee in Cameroon (Fowler and Sommer 2007, Morgan and Abwe 2006). Similarly it is known to occur only in certain populations of western chimpanzees where the distribution of this trait is regarded as a product of cultural processes (Boesch *et al.* 1994). Bark stripping occurs frequently in the manufacture of tools in GGNP and also in specific populations of western chimpanzees (Fowler and Sommer 2007). Since the characteristics of plants are unlikely to differ so as to prevent bark stripping in one location and not another it is believed to be a cultural variant (Fowler and Sommer 2007).

The Nigeria-Cameroon chimpanzee has also been heard using the buttresses of trees to create acoustic signals by drumming them with their hands and feet (Sommer *et al.* 2004). This behaviour is believed to serve the purpose of inter-party communication (Arcadi *et al.* 1998). This behaviour is present in all nine of the populations that were examined by Whiten *et al.* (2001). Evidence for self-medication has also been noted in the Nigeria-Cameroon Chimpanzee (Fowler *et al.* 2007). Un-masticated sharp edged leaves found in the faecal remains are thought to be attempts to remove parasites through induced vomiting (Fowler *et al.* 2007).

Threats and conservation

Chimpanzees are classified as Endangered by the IUCN red list (Oates *et al.* 2013). The rapid decline in abundance in recent history has prompted calls for this classification to be amended to Critically Endangered (Walsh *et al.* 2003). In the last decade, three of the five sub-species have suffered dramatic declines in population size (Walsh *et al.* 2003, Campbell *et al.* 2008, Greengrass 2009). Habitat loss and hunting are often cited as the most serious threats (Walsh *et al.* 2003, Morgan *et al.* 2011) whilst disease is a rapidly growing concern (Walsh *et al.* 2003, Pusey *et al.* 2008). Threats are interrelated, for example, commercial logging facilitates hunting by constructing roads that increase accessibility into remote forest regions. In turn the increase in human presence raises the possibility of disease transmission (Wilkie *et al.* 2000, Kormos 2003, Pusey *et al.* 2008). I summarize below the main threats that face chimpanzees in the wild today, the conservation strategies in place or proposed to mitigate these threats and mention their relevance to the Nigeria-Cameroon chimpanzee.

Habitat loss

Chimpanzees depend on the use of forests for the provision of food and the use of trees as nesting sites (Balcomb *et al.* 2000). Across Africa forests are being removed chiefly for the production of timber and the conversion to agricultural land (Kormos 2003, Laporte *et al.* 2007). These practices threaten chimpanzee populations directly by the immediate loss of the forest and indirectly by fragmenting the remaining habitat (the effects of fragmentation are discussed in more detail in Chapter three). Farming of cattle is particularly prevalent in Nigeria, especially in Taraba State (the location of this study) due to the presence of nomadic pastoralists that encourage the destruction of forest for the conversion to grassland for grazing cattle (Figure 1.2, Morgan *et al.* 2011). Pastoralists, as well as removing forest, periodically burn the grasslands around forests to encourage new growth of the grasses which damages the forest edge (Figure 1.3 and Figure 1.4). In rural regions of Taraba State slash and burn agriculture is a common practice where fertile forests are removed to plant crops such as sugar cane and corn (Figure 1.5).

In Western Equatorial Africa, which includes part of the range of the Nigeria-Cameroon chimpanzee, more than 50% of remaining chimpanzee habitat falls within logging concessions in contrast to 17% located in protected areas (Morgan and Sanz 2007). In Southwest Nigeria commercial timber companies are well established and supported by the

Figure 1.2: Pastoralist herding cattle on the southwest border of Gashaka Gumti National Park. Photo taken 23rd of April 2012 by Alexander Knight.

Figure 1.3 (left): Local farmer burning the dry grass to encourage new growth in a region between Gashaka Gumti National Park and Ngel Nyaki forest reserve. Photo taken 9th of February 2012 by Alexander Knight. **Figure 1.4** (below): Grasslands left to burn uncontrollably overnight near Gashaka Gumti National Park. Photo taken 17th of March 2012 by Alexander Knight.

government (Greengrass 2009). Rising timber production sourced from forests in southwest Nigeria is correlated with a sharp decline in chimpanzee abundance (Greengrass 2009). Logging in Nigeria is often succeeded by the creation of agricultural lands which results in the permanent loss of chimpanzee habitat (Morgan *et al.* 2011). Several forest reserves in Nigeria have been removed for oil palm and rubber plantations (Morgan *et al.* 2011). Timber extraction alters the habitat of chimpanzees by removing food sources and fragmenting populations (Morgan and Sanz 2007). Chimpanzees may be particularly susceptible to logging due to their territorial nature (Arnhem *et al.* 2007). When chimpanzees are displaced by human disturbance and forced to move into neighbouring community home ranges they may be met by aggressive conspecifics and mortality levels are exacerbated by intercommunity conflict (White and Tutin 2001, Matthews and Matthews 2004).

Conservation strategies to minimize the effect of the logging have centred on encouraging logging practices that mitigate their impacts (Morgan and Sanz 2007). Selective or Reduced Impact Logging practices (RIL) remove only a limited quantity of trees

Figure 1.5: Sugar cane farm on the edge of the forest at Akwaizantar. Photo taken on the 27th of January 2012 by Alexander Knight

per hectare. Collateral damage is also minimized by building bridges instead of damming watercourses, using smaller equipment and constructing narrower roads (WWF 2013). Currently logging concessions certified by the Forest Stewardship Council (FSC) are potentially the least detrimental to ape populations (Morgan *et al.* 2013, WWF 2013). To obtain FSC certification logging companies must adhere to a number of principles that are designed to promote the rights of workers and indigenous people, improve working conditions and mitigate environmental impacts (Morgan *et al.* 2013). Included in these is a requirement to assess the conservation status and vulnerability of species residing within logging concessions, identify threats to these species and adopt measures to limit the impact of logging on these species (Morgan *et al.* 2013). FSC logos are branded on products made from FSC certified concessions to enable consumers in choosing items made from responsibly sourced materials (Figure 1.6). Evidence for the effectiveness of RIL and FSC compliant concessions is beginning to emerge (WWF 2013). Clark *et al.* (2009) found that if responsible logging is practiced then species can recover faster and secondary forest can extend conservation areas.

Hunting

Chimpanzees are hunted for personal consumption, sale in the bushmeat trade and for use in traditional medicines (Sutton and Anderson 2005, Morgan *et al.* 2011). The growth in the bushmeat trade in Nigeria and Cameroon has been attributed to the rapidly expanding human population, the ease of access to arms and improved infrastructure for transporting bushmeat to urban markets (Morgan *et al.* 2011). The threat of the bushmeat trade has become so severe it has led to the establishment of the Bushmeat Crisis Task Force (www.bushmeat.org). Among its responsibilities the taskforce is charged with building awareness, identifying and implementing solutions to the bushmeat crisis and strengthening policies regarding the crisis.

Figure 1.6: FSC logo

Hunting and bushmeat are frequently linked to other extractive industries located in or near forests. In Gabon the presence of the oil industry resulted in the creation of a town inside a protected area which in turn led to the sale of large quantities of bushmeat in the local markets (Thibault and Blaney 2003). The construction of mines in biodiverse regions is often accompanied by the influx of professional hunters who provide food for workers (Sutton and Anderson 2004). The mining and oil industries are usually controlled by large companies that have the capacity to mitigate environmental impacts if they choose, enabling the opportunity for conservation (Sutton and Anderson 2004). Valuable minerals such as gold and diamonds, however, attract illegal or freelance operatives and organisations which are unlikely to respect environmental practices (Sutton and Anderson 2004). A prime example was the exploitation of coltan, a mineral used in the construction of electronics such as mobile phones and computers, in Rwanda and the Democratic Republic of Congo (Redmond 2001). The vast workforce that the lucrative coltan mines attracted or brought with them was responsible for the decline in numerous species in the region including chimpanzees and the loss of over 8000 gorillas (Redmond 2001, Sutton and Anderson 2004).

The slaughter of chimpanzees is illegal in Nigeria and Cameroon yet despite this fact the bushmeat trade may be the greatest threat to many populations of the Nigeria-Cameroon

chimpanzee (Morgan *et al.* 2011). In western Nigeria, particularly around Gashaka Gumti National Park, local taboos about the consumption of chimpanzee meat led to earlier conclusions that hunting pressure in this region was weaker than elsewhere (Kormos 2003). Sadly it is emerging that this is unlikely to be the case. The sale of chimpanzee carcasses has been reported in a number of markets in the region (Ogunjemite and Ashimi 2010). The price of an adult chimpanzee carcass is between 15,000 and 30,000 Nigerian Naira (approximately $100 – $200 USD) and a live adolescent fetches about 10,000 Nigerian Naira (approximately $60 USD). Hunting in the region is now believed to be a well-organized enterprise sourcing chimpanzees from the national park with a single hunting session potentially removing up to ten chimpanzees at a time (Ogunjemite and Ashimi 2010, Hughes *et al.* 2011). Even rescued chimpanzees housed as far away as the Limbe Wildlife Sanctuary in Cameroon have had their origins traced back to Gashaka Gumti National Park in Nigeria (Ghobrial *et al.* 2010).

Disease

Diseases inflicting chimpanzee populations have been recorded as far back as the 1960s when Jane Goodall began observations at Gombe, Tanzania (Pusey *et al.* 2008). The list of pathogens that chimpanzees are susceptible to has been growing since then. In Gombe, deaths have been attributed to a polio-like disease, mange and a respiratory epidemic (Pusey *et al.* 2008). The three communities studied at Gombe have responded differently to disease outbreaks. The Kasekela community, which has been exposed to the most human contact, has shown less of a decline in population size as compared to its unhabituated neighbouring community (Pusey *et al.* 2008). In Côte d'Ivoire at least six chimpanzees have died from anthrax infections (Leendertz *et al.* 2004). In Gabon and the Republic of Congo multiple epidemics of Ebola in humans have been linked back to chimpanzee carcasses (Leroy *et al.* 2004). Pre- and post-outbreak surveys in the region found an 88% decline in indexes of chimpanzee presence which are robust indicators of population size (Leroy *et al.* 2004). The central chimpanzee (*P. t. troglodytes*) is now believed to be the host reservoir of simian immunodeficiency virus, the precursor to the human immunodeficiency virus (Sharp *et al.* 2005). Unlike humans, however, chimpanzees do not appear to develop AIDS but it does cause premature death. One hypothesis to explain the reduced pathogenicity compared to humans is the little genetic diversity at loci affecting the major histocompatibility complex (de Groot *et al.* 2002). This has been interpreted as evidence of a selective sweep in ancient populations resulting in natural immunity. To date Ebola has not been detected in the Nigeria-Cameroon chimpanzee but anthrax and malaria have (Morgan *et al.* 2011) although there is no data on their impact in wild populations.

The sources of anthrax and Ebola are still unknown but are believed to come from natural hosts in the range of chimpanzees (Leendertz *et al.* 2004, Leroy *et al.* 2004). Humans as a source of infection to great apes has been suspected since gorillas succumbed to the measles that were potentially transmitted by tourists in the 1980s (Ferber 2000). There exists the possibility that all the diseases observed in the chimpanzees at Gombe, Tanzania were transmitted via humans although it has been confirmed in none of them (Wallis and Lee 1999). Habituation of chimpanzees at Gombe has involved provisioning of bananas which in many instances were handed to the chimpanzees directly from field assistants and researchers which could have facilitated direct transmission (Wallis and Lee 1999). Similarly in Côte d'Ivoire, numerous outbreaks of a respiratory disease followed habituation efforts (Köndgen *et al.* 2008). Sequencing of the pathogens causing the respiratory epidemics revealed that they are closely related to strains that are present in humans implicating humans as the source of infection (Köndgen *et al.* 2008).

Inter-species transmission can occur through a number of vectors. Direct or airborne transmission is possible especially in situations where researchers or personnel work in close proximity to apes (Woodford *et al.* 2002). The viruses of influenza, the common cold, measles and tuberculosis are present in discharge from coughing or sneezing (Woodford *et al.* 2002). Rubbish and abandoned camps are additional vectors for direct transmission (Wallis and Lee 1999). Oral transmission from human waste (faecal matter or vomit) if it is deposited in chimpanzee habitat can occur (Woodford *et al.* 20002). Mosquitos and other arthropods are potential indirect vectors for diseases such as malaria and influenza (Woodford *et al.* 2002). Reducing the rate of infection from humans to chimpanzees in long term study sites has involved the implementation of quarantine, vaccination and research protocols (Pusey *et al.* 2008). Tourists, employees and researchers are required to wear surgical masks in some field sites to limit airborne transmission (Pusey *et al.* 2008). Enforcement of a minimum distance between chimpanzees and people has been attempted but is difficult to maintain especially in research situations where chimpanzees approach the researchers in thick vegetation that prevents them from retreating (Pusey *et al.* 2002).

This study

The goal of this study was to examine several aspects of the population genetics and population biology of the Nigeria-Cameroon chimpanzee at seven sampling locations in the south of Taraba State, Nigeria. Three of the sampling locations are within GGNP and two are situated just outside the southern boundary of GGNP. The final two sampling locations are found within Ngel Nyaki forest reserve, at each of the two forest fragments inside the reserve. Ngel Nyaki forest reserve was the focus of the study and the principal goal was to

determine if the community of chimpanzees at Ngel Nyaki forest reserve has become isolated from the chimpanzees at GGNP using microsatellite loci extracted from non-invasive sources of DNA. In Chapter two, the methods used to extract and amplify the DNA and the protocols used to confirm the genotypes are outlined. Chapter three examines the population structure of the chimpanzees among the regions sampled in this study, particularly addressing the question as to whether the chimpanzees at Ngel Nyaki forest reserve are isolated from the chimpanzees at GGNP. Chapter four investigates patterns of sex biased dispersal in the Nigeria-Cameroon chimpanzee. In Chapter five, population viability analysis is used to determine the fate of the chimpanzees at Ngel Nyaki forest reserve under a range of management scenarios. Chapter six summarizes the conclusions of the study and presents a conservation strategy to ensure the viability of the population of chimpanzees at Ngel Nyaki forest reserve.

Chapter 2 Methods

Study site

Chimpanzee faecal samples were collected from eight locations in Taraba State, Nigeria (see Figure 2.1). Taraba State is located on the eastern border of Nigeria adjacent to Cameroon. At its lowest point the land is approximately 200 metres above sea level reaching up to Nigeria's highest point 2,419 metres above sea level at the peak of Gangirwal, Nigeria's highest mountain, on the border with Cameroon. Located in the east portion of Taraba State, is Gashaka Gumti National Park (GGNP) measuring 6731 km^2 in size (Sommer and Ross 2011). Along the southern and eastern borders of the park the land rises sharply up to the Mambilla plateau sitting at approximately 1600 metres above sea level. The plateau runs along the eastern border of Nigeria into the mountainous highlands around Gangirwal. Ten kilometres south of the border of GGNP, up on the Mambilla plateau is Ngel Nyaki forest reserve. The reserve is 43 km^2 in area but the forest covers just 7 km^2 and is divided into two fragments, Ngel Nyaki forest and Danko forest.

The region has distinct wet (May to October) and dry (November to April) seasons. The average yearly rainfall is approximately 1935 mm (range 1683 – 2337 mm, Sommer and Ross 2011). Temperature in the lower regions of the national park averages between 20.9°C and 32.2°C but up on the Mambilla plateau the temperature is slightly cooler with average temperatures ranging between 16°C and 26°C (Chapman and Chapman 2001)

The region is recognised internationally for its biodiversity. The World Wildlife Fund – UK was instrumental in developing GGNP to protect its unique fauna and flora (Barnwell 2011). The region is part of Conservation International's Guinean Forests of West Africa biodiversity hotspot (Conservation International 2013) and one of Birdlife International's important bird areas (Birdlife International 2013).

Sample collection

To investigate population structure and dispersal patterns DNA was amplified from microsatellite loci extracted from faecal material. All faecal samples were collected between January 2012 and April 2012. Faecal samples were located by searching forests until

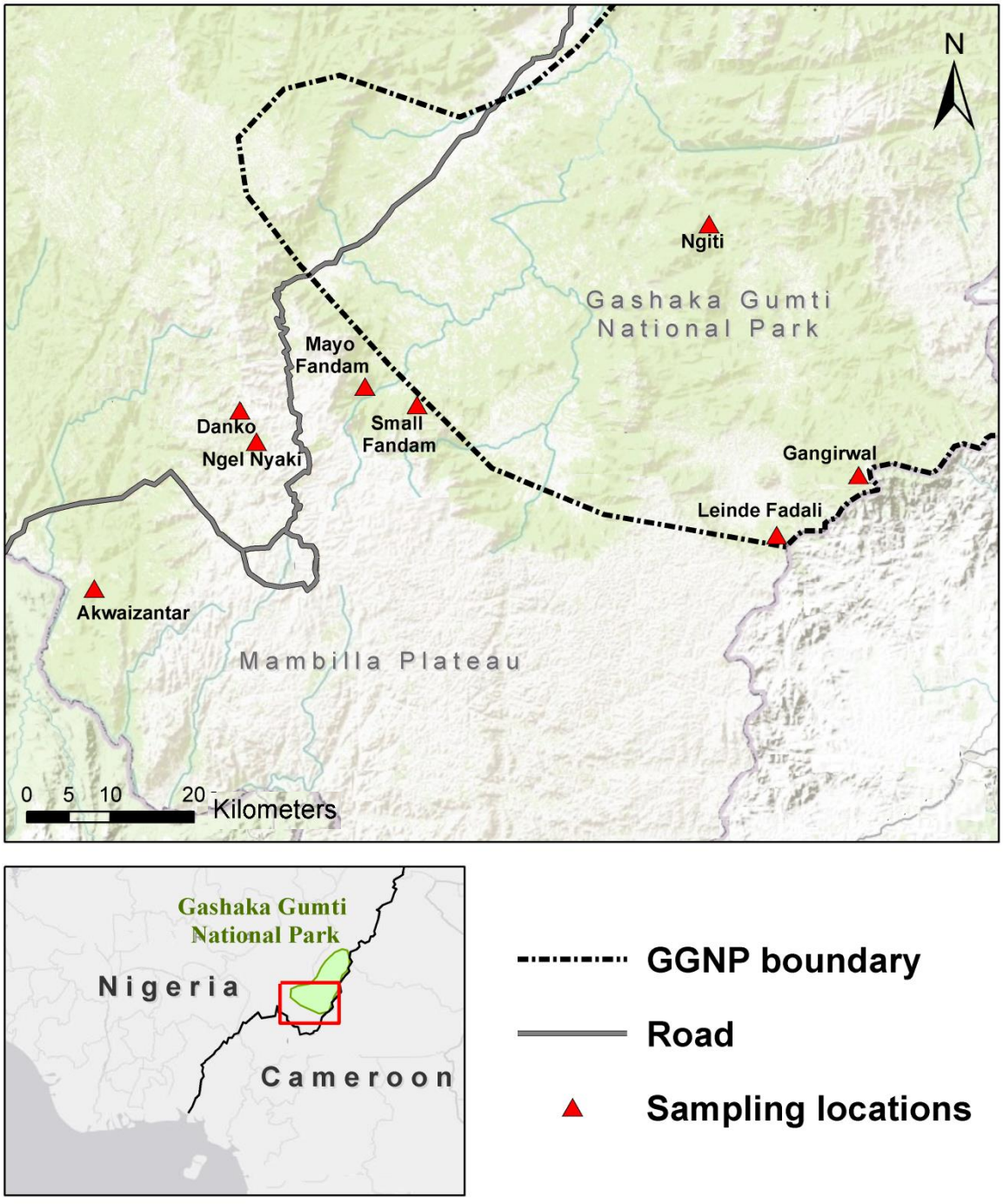

Figure 2.1: Map of the sampling locations in this study in Taraba State, Nigeria and the location of Gashaka Gumti National Park.

chimpanzees were heard, then following them for as long as possible or until nightfall. Upon daybreak of the following day, the area where the chimpanzees were tracked to the day before was searched for fresh nests. When fresh nests were located, the ground beneath was searched for faecal material. Nests were deemed to be fresh by observing the colour and age of the leaves in the nest. If it was not possible to follow the chimps until nightfall, the following day we searched in the direction of the last known movement, looking for nests or until we heard them again and continued to follow them once more. On occasion fresh faecal matter was encountered sporadically whilst tracking chimpanzees throughout the forest, this was also collected.

If there was any doubt about the age of the nest above the faecal material or the faecal material was found opportunistically while tracking, its freshness was judged by moistness. Desiccated material was ignored. All faecal material was collected whilst wearing disposable surgical gloves. 25 ml pots were prepared by filling them with 10 ml of RNAlater (Ambion). RNAlater acts as a storage buffer to protect DNA from degradation before freezing it. The faecal material was collected using an internal scoop on the lid of the pots. Each pot was labelled with a number and a code detailing the forest it was collected in, the date and a unique identifier. The pots after being closed were sealed with parafilm to help prevent leakage. Each faeces was sampled twice and stored separately, one sample being labelled 'a' and the other 'b', except for one faeces which there was not sufficient material to collect two samples from. All samples were placed in a freezer at a temperature approximately between -4°C and -17°C within seven days of being collected. A total of 85 samples were collected from all the locations combined.

On the 21st of February 2012, all the 'b' samples that had been collected up to that point were couriered to New Zealand by DHL. All the remaining 'a' samples and every sample collected after that point were transported back to New Zealand personally. The approximate time for the samples to be couriered was ten days and they were most likely stored at room temperature for the duration. Samples transported personally took seven days from the freezer in Nigeria to the freezer in New Zealand, all but one evening of this journey the samples were stored in fridges overnight, and transported with cool packs during the day. Samples were stored at -20°C upon arrival in New Zealand.

Microsatellite loci selection

In this study, previously designed primers for fourteen loci were used. Thirteen of these were provided by Dr Katherine Gonder of the University of Albany and one, used for determining the sex of the chimpanzee that the samples came from, was taken from Bradley *et al.* (2001). From these fourteen loci nine were used in the final analyses. The remaining

five were excluded because one failed to amplify any DNA, one had persistent stuttering peaks, one consistently amplified a peak that was suspected to be an artefact and hindered the validation of the true genotype and two were monomorphic. Details on the primer names, the chromosome they are located on, number of base pairs in the repeat, annealing temperatures, primer sequences with fluorescent dyes attached to the primers and whether they were included or excluded from the final analysis are given in Table 2.1.

Preventing contamination

Non-invasive DNA sources are often degraded and the target DNA might only exist in very low quantities (Taberlet *et al.* 1999). Due to these facts amplification of non-invasive DNA is highly susceptible to sporadic contamination either from DNA in the environment, the researcher or cross contamination between samples. The multiple tubes procedure was

Table 2.1: Details of loci used in this study. 'Ch #' is the chromosome number that the loci are located on. 'Base pair repeat #' is the number of base pairs repeated in the microsatellite, *exact lengths of fragments amplified at the amelogenin locus are quoted not base pair length, 'Ta' is annealing temperature. 'Included/Excluded' refers to whether it was included or excluded in the analyses and reasons for exclusion are stated in brackets. Sequence and attached dye are given in final column.

Name (Forward, *Reverse*)	**Ch. #**	**Base pair repeat #**	**Ta**	**Included/ Excluded (*Reason for Exclusion*)**	**Fluorescent dye-Forward Primer, *Reverse Primer***
Amelogenin	X/Y	106/112*	60	Included	PET-CCC-TGG-GCT-TGT-AAA-GAA-TAG-TG *ATC-AGA-GCT-TAA-ACT-GGG-AAG-CTG*
Mfd23 *D16S265*	16	2	57.8	Included	VIC - CCA GAC ATG GCA GTC TCT A *AGT CCT CTG TGC ACT TTG T*
G00-228-893 *D4S1652*	4	4	57.8	Included	6FAM - AAT CCC TGG GTA CAT TAT ATT TG *CAG ACA TTC TTT ATT CTT TAC CTC C*
G00-218-317 *D9S303*	9	4	57.8	Included	NED - CAA CAA AGC AAG ATC CCT TC *TAG GTA CTT GGA AAC TCT TGG C*
G00-364-824 *D20S470*	20	4x4x4x2	57.8	Excluded (Failed to amplify)	PET - CCT TGG GGG ATA TAG CCT AA *TGA GTG ACA GAG TGA TAC CAT G*
G00-364-803 *D11S1984*	11	4	57.8	Excluded (Monomorphic)	PET - GGG TGA CAG AGC AAA ATT CT *ACA CCT GGA TCT TGG ACT CA*
G00-364-834 *D7S1809*	7	4	57.8	Excluded (Artefact)	6FAM - AGG CAA GAG CAG TAG CAA GA *TCC ACT TTA AAT CAG CAG CC*
Mfd3 *APOA2 (D1)*	1	2	57.8	Excluded (Stutter)	VIC - GGT CTG GAA GTA CTG AGA AAA *GAT TCA CTG CTG TGG ACC CA*
ATA28F03 *D4S3248*	4	4	57.8	Included	NED-TTC AGG AGT TTA GCT TTC TAT GC *CTA CAC CAT CAG TAC TCA CTA GGC*
GATA164B08P *D3S4545*	3	4	58	Included	VIC-CTG TGA TCA CAC CAC TGC AG *TGG GGT ATC CTG TGT CAG AGC*
GATA43C11 *D7S1804*	7	4	58	Included	6FAM-TTC AAG TGG TTG GGT TCA CT *TGG GTC TAG TCC AGT GGT GT*
GATA14E09 *D8S2324*	8	4	58	Included	NED-TGA AAA CAT AGT ACA ATG AAC ATC C *GTC ATA ATA TCT GCC AAT GAT TG*
GATA50G06 *D15S643*	15	4	58	Included	PET-ATA CCT GGA GTC CTT GGT CC *AAC AGC TTT AAA ACC TCA ATG C*
ATA27A06P *D12S1042*	12	3	57.8	Excluded (Monomorphic)	PET-TAT GAC GGT GCA CCA CAT AC *AAC CTG CAT GTT CTG CAT AT*

employed to identify and mitigate errors associated with contamination (Navidi *et al.* 1992, Taberlet *et al.* 1996); this will be discussed in more detail below. Additionally the steps below were also taken to prevent contamination during the extraction and amplification process.

1. All reagents, samples, extraction products and equipment were handled whilst wearing surgical gloves.
2. Extractions were done in a room dedicated for that purpose.
3. Following completion of the extractions and cleaning with bleach the room was used for the preparation of PCR reactions.
4. Handling of all products post-PCR was done in a separate area, any equipment that was returned to the pre-PCR room was soaked in 10% bleach before being used again.
5. The surfaces in the pre-PCR/extraction room were bleached at the beginning and end of each day with a 10% bleach solution.
6. Only filtered pipette tips were used during the entire study.
7. Multiple negative controls were run during each PCR to check for reagent contamination.
8. All field assistants and people who worked in the laboratory were typed for the same microsatellite loci to check for human contamination.

DNA extraction

Two DNA extraction methods were employed. A Qiagen mini stool kit was used to extract the majority of samples. A CTAB method was also utilized (Zhang *et al.* 2006). Exact protocols for each method are outlined in the appendix. Extractions were stored in a freezer at -20°C.

DNA amplification

For the majority of amplifications each locus was amplified separately, in a number of instances a ‘two-step’ multiplex amplification was trialled. All extractions were amplified by polymerase chain reaction in an Eppendorf MasterCycler ep PCR machine and genotyped on an Applied Biosystems 3130x Genetic Analyzer.

When each locus was amplified separately the total reaction volume was 15 µl, consisting of 1.5 µl of Bioline 10x buffer, 9.58 µl of PCR grade water, 0.02 mM of each dNTP, 1.5 mM of $MgCl_2$, 2.6 mM of each primer, 0.6 units of Biotaq polymerase (Bioline) and 2 µl of template DNA .

Four amplification protocols were used in the PCR. The first protocol used had an initial denaturation at 95°C for 3 minutes then 45 cycles of 95°C for 30s, 55°C for 30s, 72°C for 30s followed by a final extension time of 72°C for 30 minutes. To reduce the effect of stuttering this protocol was modified. The modified protocol was exactly the same as the first except the annealing temperate was increased to 57.8°C for nine loci and 58°C for four loci (see Table 2.1). This second protocol was used for the majority of amplifications. A third protocol was employed to reduce the presence of persistent stuttering in some samples. This protocol consisted of an initial denaturation of 95°C for 3 minutes then 40 cycles of 95°C for 35s, 58°C for 27s, 72°C for 25s and a final extension time of 72°C for 25 minutes. The fourth protocol was used only for the amelogenin primer to determine the sex of the chimpanzee that each sample came from and was taken directly from Bradley *et al*. *(*2001). This protocol consisted of an initial denaturation of 95°C for 3 minutes then 45 cycles of 95°C for 30s, 60°C for 30s, 72°C for 30s followed by a final extension time of 72°C for 30 minutes.

When the 'two-step' multiplexing was trialled, samples were initially amplified for five loci simultaneously. The total reaction volume for this initial amplification was 20 µl, consisting of 7.74 µl of PCR grade water, 1.5 µl of Bioline 10x buffer, 0.02mM of each dNTP, 1.5mM of $MgCl_2$, 1.95 mM of each primer, 0.8 Units of Biotaq polymerase (Bioline) and 5 µl of template DNA. This multiplex extraction was diluted by adding 90 µl of PCR water to 10 µl of multiplexed extract. Each diluted multiplex extraction was then subsequently amplified for each locus individually using the first reaction mixture and the second amplification protocol detailed above.

All amplifications were prepared for genotyping in plates with 12 µl of Hidi™ formamide (Applied Biosystems) and 0.3 µl of Genescan™ 500 Liz® size standard (Applied Biosystems) per well. Up to four amplified loci with different fluorescent dyes where placed in each well in volumes of 1 µl each. Genotypes lengths were scored manually using SoftGenetics GeneMarker Version 2.4.0.

Classifying heterozygotes and homozygotes

As well as being susceptible to sporadic contamination, low quality DNA is also prone to false alleles and allelic drop out (Dewoody *et al.* 2006). The multiple tubes procedure,

where each extraction is divided into separate tubes and then subsequently amplified, was employed in this study to mitigate the effect of these confounding factors. The multiple tubes procedure allows for the identification of contamination, false alleles and allelic drop out by building consensus genotypes from multiple amplifications, on the assumption that the true genotype is amplified the most frequently (Pompanon *et al.* 2005).

The number of amplifications required to be certain of a correct genotype has been debated (Navidi *et al.*1992, Taberlet *et al.* 1996, Miller *et al.* 2002). Taberlet *et al.* (1996) showed that the confidence associated with a specific number of amplifications is a function of the amount of DNA in the sample extract. Thus more amplifications are required for very low amounts of DNA. Without applying a specific assay that can measure the amount of target DNA, such as quantitative PCR that is relatively expensive, the quantity of DNA is unknown. Whilst the number of amplifications can always be increased to gain greater confidence in the consensus genotype this can be unnecessarily costly and time consuming. To prevent extraneous amplifications and unnecessary costs a sequential probability method (Gagneux *et al.* 1997) was used to determine the required number of amplifications to ascertain a probability of <0.01 that each genotype has been incorrectly assigned. The procedure has two steps, one for identifying the number of amplifications required for homozygotes and one for heterozygotes.

Determining a probability of <0.01 for a false homozygote

A 'training' data set was compiled using a subset of samples that were confirmed as heterozygotes for each locus. For a sample to be confirmed as a heterozygote each allele had to be amplified at least three times each. For two of those three amplifications both alleles had to be amplified together as a visible heterozygote. The mean number of samples for each locus used in the training sets was 21.625 (range 10-29) and the mean number of amplifications included was 109.625 (range 42-187).

Using these training sets for each locus, instances of allelic dropout were counted and the allelic dropout rate per locus was calculated (see Table 2.2). To determine a per sample probability of <0.01 of a false homozygote, a Bonferroni correction (Bland 2000) was applied to account for multiple testing. The homozygosity for each locus was estimated in GenAlex 6.5 (Peakall and Smouse 2006) using the formula for Hardy Weinberg Equilibrium to obtain an indication of the degree of correction required. This estimate of homozygosity was then multiplied by the number of samples to give an estimate of the number of homozygotes that might occur for that locus. For example, the estimated homozygosity for locus G00-228-893 was 0.178, multiplied by 63 (note that the number of samples here is fewer than the total number collected because it had become apparent when compiling the

training sets that some of the samples had degraded such that no DNA could be extracted or amplified) which yielded an expected number of 11.214 homozygotes, to be conservative this was rounded up to twelve. The necessary probability that had to be obtained to give a per sample probability of <0.01 is equal to 0.01 divided by 12, which is equivalent to 0.0008. Therefore using the sequential probability formula:

$$\frac{P(false\ homozygote) = 0.01}{Ho * S} > a * (a/2)^{n-1}$$

Where *Ho* is the estimated homozygosity, *S* is the number of samples, *n* is the number of reactions, and *a* is the allelic dropout rate. When *n* is sufficiently high so that the value on the right side of the equation is less than the value on the left then *n* is the number of amplifications required to obtain a per sample probability of 0.01 that the sample is a false homozygote. For the example of locus G00-228-893, *n* has to equal 3 before the probability is less than 0.0008, thus 3 amplifications are required to have a probability of <0.01 that the sample is a false homozygote. The number of required amplifications for each locus is given in Table 2.2.

This method assumes that each allele has an equal probability dropping out. Given that the larger allele in a heterozygote has been noted to dropout more frequently (Wattier *et*

Table 2.2: Details of the training sets used to obtain a P<0.01 of scoring a false homozygote. 'ADO rate' is the allelic dropout rate. 'X^2 test' is the Chi square test of independence test statistic and its associated P value in brackets below for determining the relationship between dropout rate and allele size. 'Estimated Ho' is the estimated homozygosity. 'Replicates required…' is the number of successful amplifications required.

Primer (forward/ *reverse)*	Total number of amplifications used in training set	Frequency of allelic dropout	ADO rate	X^2 test (P value)	Estimated Ho	Replicates required for P<0.01 (after correction)
Mfd23/ *D16S265*	137	18	0.131	6.3778 (0.3822)	0.618	4
G00-228-893/ *D4S1652*	118	9	0.076	4.2778 (0.233)	0.177	3
G00-218-317/ *D9S303*	98	9	0.091	2.2611 (0.812)	0.202	3
ATA28F03/ *D4S3248*	42	8	0.190	5.1333 (0.149)	0.25	4
GATA164B08 P/ *D3S4545*	112	7	0.062	4.958 (0.1749)	0.094	3
GATA43C11/ *D7S1804*	95	7	0.073	4.55 (0.4732)	0.315	3
GATA14E09/ *D8S2324*	120	8	0.066	6.107 (0.1913)	0.296	3
GATA50G06/ *D15S643*	113	15	0.085	2.057 (0.7252)	0.177	3

al. 1998, Dewoody *et al.* 2006), a chi square test of independence was used to ascertain whether this occurred at any particular locus. There was no significant relationship between allele size and dropout rate at any locus (Table 2.2).

Determining a probability of <0.01 for a false heterozygote

A second 'training' data set was established using the genotypes from the first set plus any homozygotes that had been identified given the new criteria for homozygotes and extra heterozygotes that had been amplified at the time of compiling the training set. The mean number of samples included in the new training set was 29.25 (range 15-35) and the mean number of amplifications was 139.625 (range 76-199).

I counted the number of false alleles and contaminated negative controls and divided this by the total number of amplifications and negative controls to estimate a false allele plus contamination rate. Contaminated controls were included in this calculation because it is not always possible to distinguish the difference between a false allele and a contaminated sample. A Bonferroni correction (Bland 2000) was applied again to calculate the necessary probability required to gain a per sample probability of <0.01. Thus:

$$\frac{P(false\ heterozygote) = 0.01}{He * S} > (fa + c)^n$$

Where *fa* + *c* is the locus specific false allele and contamination rate, *n* is the number of amplifications, *He* is the estimated heterozygosity and *S* is the number of samples. Again when *n* is sufficiently high that the value on the right side of the equation is less than the value on the left, *n* represents the necessary amount of amplifications to ensure the probability of detecting a false heterozygote is <0.01. The number of amplifications required for each locus to have a probability of less than 0.01 for a false heterozygote is shown in Table 2.3. In instances where two or more matching heterozygotes had been amplified accompanied by an amplification of a matching homozygous allele to one of the heterozygotic alleles, it was attributed to allelic dropout and counted as a successful amplification.

Sex determination

The sex of the chimpanzees was determined using the amelogenin locus (Bradley *et al.* 2001). The amelogenin primers amplify a 106 bp fragment on the X chromosome and a 112 bp fragment on the Y chromosome. Thus if the sample comes from a female the genotype will be a homozygote for the 106 bp fragment. If the sample comes from a male then the genotype will be a heterozygote for the 106 bp and 112 bp fragments. If the sample

Table 2.3: Details of the training sets used to obtain a P<0.01 of scoring a false heterozygote. '# FA' is the frequency of false alleles for that locus in the training set. '# Negative controls' is the number of negative controls. '# contaminated controls' is the number of contaminated controls. 'FA+C rate' is the combined rate when both false alleles and contaminated controls are considered. 'Estimated He' is estimated heterozygosity. 'Replicates required…' is the number of successful amplifications necessary.

Primer (forward/ *reverse)*	Total number of amplifications used in training set	# FA	# Negative controls	# Contaminated controls	FA + C rate	Estimated He	Replicates required for P<0.01 (after correction)
Mfd23/ *D16S265*	199	9	39	3	0.05	0.771	3
G00-228-893/ *D4S1652*	126	3	30	2	0.032	0.822	3
G00-218-317/ *D9S303*	135	2	48	5	0.038	0.769	3
ATA28F03/ *D4S3248*	76	2	28	0	0.019	0.596	3
GATA164B08 P/ *D3S4545*	154	8	45	4	0.052	0.913	3
GATA43C11/ *D7S1804*	167	0	53	4	0.018	0.616	3
GATA14E09/ *D8S2324*	131	6	48	1	0.039	0.715	3
GATA50G06/ *D15S643*	142	3	45	0	0.010	0.823	2

amplified only for the 106 bp fragment four times in a row it was recorded as a female. When a sample was amplified for the 106 bp fragment and the 112 bp fragment three times it was recorded as a male.

Ambiguous results

Samples were considered ambiguous if in the case of a homozygote, one amplification resulted in a heterozygote or an alternative homozygote, or in the case of a heterozygote one amplification resulted in homozygote for a third allele or a different heterozygote. If either of these scenarios occurred then at least two more amplifications were performed, if the results continued to be ambiguous then the data for that genotype was scored as missing.

Scoring genotypes

Once all genotypes had been classified as heterozygotes, homozygotes or missing data a series of genotype lengths for each locus was created. All samples were double checked and scored according to the series. All loci included in the final analysis were tetranucleotide repeats except for locus Mfd23 which was a dinucleotide repeat and the locus used to determine the sex of the chimpanzees. If the peak in the electropherogram was one base pair above or below the series then it was rounded up or down to match the series. For example in locus G00-228-893 the series was in units of four base pairs, i.e. 158, 162, 166…etc. If a peak appeared as 165 this would be scored as 166. In certain

cases the peaks consistently fell midway between two points in the series and so were scored at the midway point. For locus Mfd23, which has a dinucleotide repeat, samples were always scored along the series; there was no instance of one sample repeatedly falling midway between two series points.

Null alleles, stuttering and large allele dropout

The data were checked using MICROCHECKER (van Oosterhout *et al.* 2004) for evidence of null alleles, scoring errors due to stuttering and any evidence of large allele drop out in the final data set. No evidence was found for any one of these three potential sources of error.

Error rates

To estimate the quality of the data I estimated the mean allelic dropout rate, mean false allele rate and the observed multilocus error. High multilocus error rates can affect estimates of probability of identity, population assignment and population size estimates (Pompanon *et al.* 2005). I estimated the mean allelic dropout and false allele rate as per Broquet and Petit (2004). The multilocus error rate was calculated by genotyping approximately 12% (8/63) of the duplicate samples for as many loci as possible. Not all loci were genotyped for the duplicate samples due to samples degrading over time. All error rates are shown in Table 2.4.

The formulas for each error rate are as follows:

Mean allelic dropout rate:

$$\frac{\sum ADO}{\sum r}$$

where *ADO* is the occurrence of allelic drop out for all loci, *r* is the total number of positive amplifications for which allelic dropout could have potentially been detected in.

Mean false allele rate:

$$\frac{\sum F}{\sum r}$$

where *F* is the false allele rate for all loci and *r* is the total number of positive amplifications that false alleles could have potentially been detected in.

The multilocus error rate:

$$\frac{\sum m}{\sum d}$$

where *m* is the number of mismatched genotypes and *d* is the total number of duplicated genotypes.

Table 2.4: Error rates. Frequency of error refers to the number of times that particular error was detected. The total number of amplifications is the number of amplifications for all loci that that error could have potentially been detected in. For the multilocus error rate the total number of amplification is equal to the number of loci amplified in all the duplicate samples. The error rate is calculated from the frequency of that error divided by the total number of amplifications used.

Error type	Frequency of error	Total number of amplifications used	Error rate
Mean allelic dropout	81	835	0.097
Mean false allele	33	1130	0.029
Multilocus error	1	31	0.032

Chapter 3 Genetic Structure and Inbreeding

Introduction

Genetic diversity, inbreeding and inbreeding depression

The importance of genetic diversity to the maintenance of wild populations is now a well understood phenomenon (Frankham *et al.* 2002). In the long term, genetic diversity provides species with the evolutionary potential for adaptation to changing environments. A more immediate threat, however, is a reduction in the fitness of organisms with lower genetic diversity (Vrijenhoek 1994, Reed and Frankham 2003). It has been well established that population size is correlated with genetic diversity (Frankham 1996, Epps *et al.* 2005, Frankham 2005). Small populations lose genetic variation due to drift and the rate of inbreeding, the mating of related individuals, increases (Falconer and Mackay 1996, Reed 2005).

Inbreeding impacts populations negatively by altering the genotype frequencies, resulting in a reduction in heterozygosity in individuals and a corresponding increase in homozygosity (Hamilton 2011). Consequently, recessive or partially recessive deleterious alleles, which were once masked in heterozygotes, are exposed and heterozygotes that might have a fitness advantage decline in frequency (Charlesworth and Charlesworth 1999). When traits directly or indirectly related to fitness are adversely affected, it is known as inbreeding depression. The ubiquity of inbreeding depression in different taxa is now incontrovertible having been evidenced in fish (Vrijenhoek 1994), molluscs (Chen 1993), felids (Thornhill 1993), ungulates (Slate *et al.* 2000), primates (Charpentier *et al.* 2007), canids (Liberg *et al.* 2005), birds (Keller *et al.* 1994), invertebrates (Saccheri *et al.* 1998) and plants (Ratchke and Real 1993).

There was some initial debate as to whether inbreeding plays a significant role in species declines (Simberloff 1986, Lande 1988, Caro and Laurenson 1994, Caughley 1994). Evidence that it does affect species at a population level has been accumulating for more than two decades. In the laboratory, inbred populations of *Drosophila* show a higher probability of extinction than outbred ones (Bijlsma *et al.* 2000). Captive populations of inbred animals have higher rates of juvenile mortality than outbred lines (Ballou and Ralls

1982, Ralls and Ballou 1982a, 1982b, Ralls *et al.* 1988). Furthermore the effect of inbreeding depression is argued to be more severe in the wild (Miller 1994). Crnokrak and Roff (1999) showed the cost of inbreeding depression to be significantly higher in the wild than in the captive populations studied by Ralls *et al.* (1988).

A common argument against inbreeding depression contributing to species extinctions is that a species will be driven extinct by demographic and anthropogenic forces before genetic factors become involved (Simberloff 1986, Lande 1988). Contrary to this, Spielman *et al.* (2004) demonstrated that heterozygosity is, on average, 35% lower in threatened taxa than non-threatened taxa, a measure that is correlated with fitness (Reed and Frankham 2003). This doesn't implicate inbreeding as a cause of the threatened status, but does suggest that threatened taxa are currently suffering a loss in reproductive fitness which will contribute to their further decline. Keller and Waller (2002) point out that early scepticism (Caro and Laurenson 1994) was attributed to the inability to assign mortality to genetic causes. Their review of the subject emphasises that inbreeding does not necessarily cause mortality but frequently increases susceptibility to environmentally inflicted mortality (see Keller *et al.* 1994, Coltman *et al.* 1999 for examples). Lande (1988), an often cited critic, also later conceded that the interaction of genetic factors with demographic and anthropogenic forces will be sufficient to drive species into extinction 'vortexes' (Lande 1998).

Fragmentation, diversity and population persistence

Habitat fragmentation is one such anthropogenic force. The division of continuous habitat into a collection of smaller habitat patches has the potential to isolate resident organisms. This process can reduce the effective population size and interrupt the dispersal behaviour of species occupying the habitat (Fahrig and Merriam 1994). Populations that become isolated are expected to become genetically differentiated due to drift and local selection if the rate of mutation is negligible and gene flow is sufficiently low (Frankham *et al.* 2002, Hamilton 2011). Genetic structuring of populations in this manner is common in both naturally (Templeton *et al.* 1990, Eriksson *et al.* 2004) and anthropogenically fragmented landscapes (Gerlach and Musolf 2000, Epps *et al.* 2005, Goossens *et al.* 2005a). Smaller and more isolated populations often exhibit lower genetic variation (Dixon *et al.* 2007, Dixo *et al.* 2009). As a consequence of the reduced effective population sizes, sub-populations become exposed to the negative genetic effects of small populations: inbreeding, the accumulation of deleterious alleles and the loss of diversity (Frankham 2005).

Despite this intuitive theory, the dynamics of population persistence in light of fragmentation and genetic stochasticity are not simple and there are a number of other key

elements. Species specific characteristics can influence susceptibility to fragmentation. Demographic studies have linked ecological specialism, rarity, trophic status, abundance, fluctuations in abundance and competition to varying responses to fragmentation (Davies *et al.* 2000, Henle *et al.* 2004). Genetic differentiation between sub-populations can also be influenced by ecological specialism, generation time and fecundity (Brouat *et al.* 2003, Srikwan and Woodruff 2000 cited by Keyghobadi 2007). Reed's (2004) theoretical modelling of extinction risk calculates that populations of less than 1000 will always fare better in a continuous population and population growth rate will be correlated with the probability of persistence. The landscape itself may also have a considerable effect. Johansson *et al.* (2005) discovered that genetic variation in an anuran species was negatively impacted by agricultural fragmentation in the southern end of their study site in Sweden. In the north, however, agricultural fragmentation was associated with higher degrees of genetic variation. They proposed that the greater variation in the agricultural lands found in the north might be more conducive to anuran life histories.

Above all else, gene-flow and recolonization rates are potentially the most critical parameters in determining long term persistence in fragmented populations (Templeton *et al.* 1990, Hanski 1998). Gene flow mitigates the negative consequences of small population sizes. Theoretical and experimental evidence imply that as few as one migrant per generation is enough to prevent the detrimental effects of inbreeding and maintain genetic variation (Newman and Tallmon 2001, Couvet 2002). Templeton *et al.* (1990) postulated that without sufficient dispersal to recolonize patches after local extinction, an 'extinction ratchet' would steadily raise the global extinction risk. The fewer occupied patches in a metapopulation, the lower the probability is of population persistence in the landscape as a whole (Hanski 1998)

Taraba State, Nigeria and the Nigeria-Cameroon chimpanzee

The Mambilla plateau is located on the eastern flank of Nigeria in Taraba State bordering Cameroon. At the base of the Mambilla Plateau is Gashaka Gumti National Park (GGNP) which is the largest protected region in the range of the Nigeria-Cameroon chimpanzee (Sommer *et al.* 2004, Morgan *et al.* 2011). The region is being deforested due to agriculture, palm oil plantations and cattle herding (Morgan *et al.* 2011). Pockets of forest still remain on the periphery, and to a much lesser degree outside of the park. Despite being a national park human settlements have been allowed to remain in the park. Inhabitants farm crops within allocated areas and keep livestock. Cattle farming is prevalent throughout much of the region, not just by inhabitants of the national park but for a nomadic tribe, the Fulani, it is their principal form of livelihood. Cattle erode the forest edge and

prevent new growth from establishing. Furthermore, shepherds periodically burn the landscape to encourage the growth of new grass (Sommer *et al.* 2004, Morgan *et al.* 2011). Swathes of fire often breach the forest edge destroying the forest further.

Aside from GGNP the only forest in this region that receives any formal protection in the form of regular patrols is Ngel Nyaki forest reserve. Deforestation in the region is heavy (Oates *et al.* 2003, Morgan *et al.* 2011); therefore it is not inconceivable that in the near future only GGNP and Ngel Nyaki forest reserve will contain the last remaining suitable habitat for chimpanzees in this part of Taraba State. Nigeria-Cameroon chimpanzees have a number of life history traits that would suggest they are highly susceptible to the processes of fragmentation: long generation times, low fecundity and very slow population growth rates (Reed 2004, Srikwan and Woodruff 2000 cited by Keyghobadi 2007). They are, however, highly adaptable and have the potential for long distance dispersal.

I examined the level of genetic differentiation between groups of chimpanzees living in different regions of GGNP, on the periphery and in Ngel Nyaki forest reserve outside the park. The purpose was to determine whether the community at Ngel Nyaki forest reserve is isolated from GGNP and to compare levels of inbreeding in the various communities. Ngel Nyaki forest reserve is located about 10-15 km south west of the park boundary. Between Ngel Nyaki forest reserve and the park boundary there exist very small remnant forests patches but the majority of the landscape is now agricultural land. A road runs between Ngel Nyaki forest reserve and the park and human settlements are located frequently along the road. There do however remain sections of road that have no dwellings and could potentially allow chimpanzees to cross unnoticed.

Methods

Sample collection

A total of 85 samples were collected from eight forest fragments in Taraba State. Two of these fragments, Ngel Nyaki and Danko are located in Ngel Nyaki forest reserve, 10-15 km southwest of GGNP (see Figure 3.1). One fragment, Ngiti, is located near the centre of GGNP. Leinde Fadali and Gangirwal are both on the eastern border of the park but located within the park boundary. Small Fandam and Mayo Fandam are located approximately one and three kilometres, respectively, south west of the boundary of GGNP (see Figure 3.1). The land between Small Fandam, Mayo Fandam and GGNP is sparsely inhabited and it is unlikely that it acts as a biological barrier to chimpanzees therefore these locations have been included as part of GGNP in the analyses. Akwaizantar is located approximately 45 km south west of GGNP, however, none of the samples collected at

Akwaizantar amplified sufficient DNA to be included in the analyses. From the 85 samples collected, DNA was successfully extracted from 63 of them (see Chapter two for more details of DNA extraction, amplification and genotyping). The samples were typed for eight polymorphic microsatellite loci using the multiple tubes procedure (see Chapter two for more detailed information). Thirty seven of the samples had unique genotypes for five or more microsatellite loci and were included in the following analyses. The number of samples collected and the number of unique genotypes obtained from each location are shown in Table 3.1.

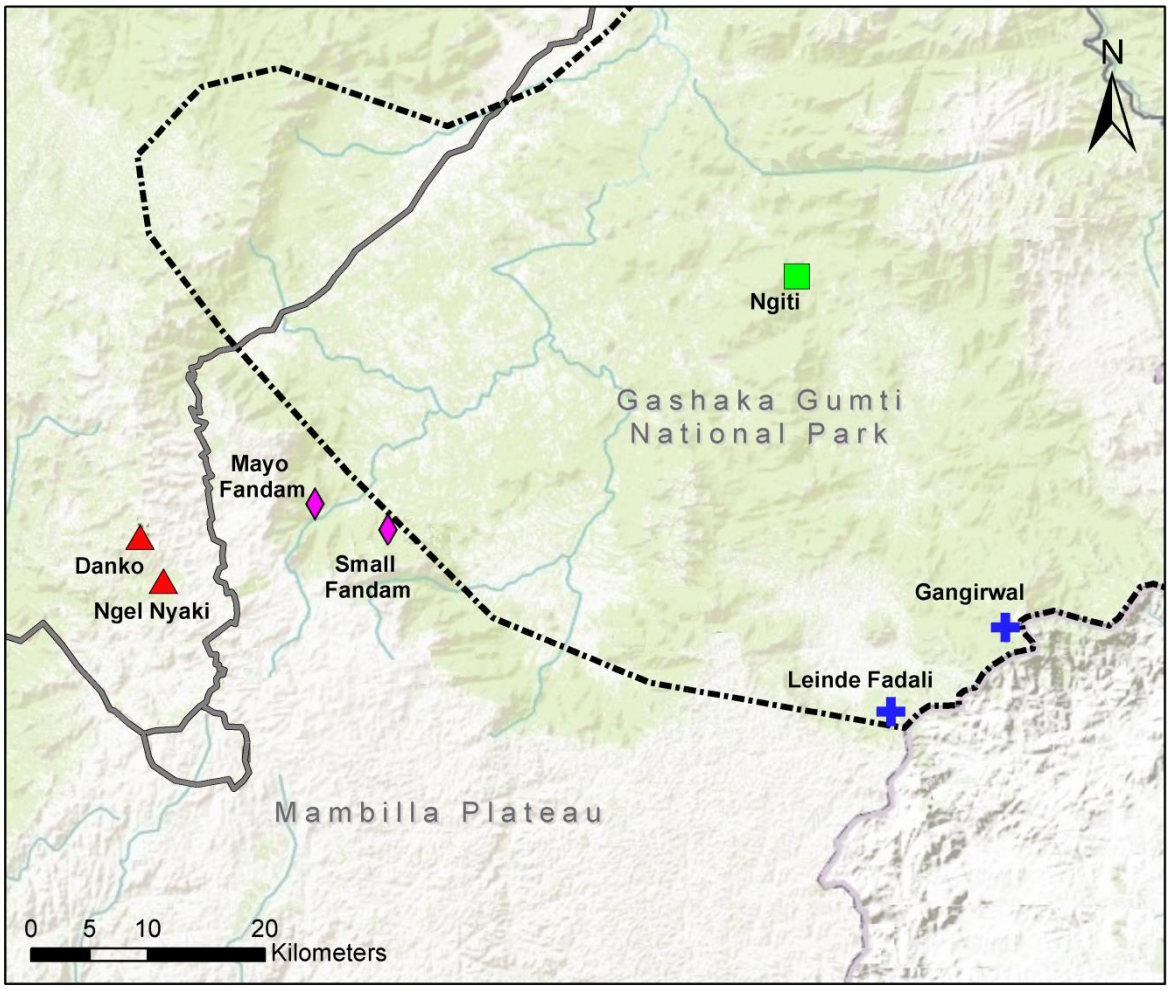

Figure 3.1: Map of the sampling locations used in the analyses presented in this chapter. Sampling locations are symbolized by the regions they were grouped by in the analysis of molecular variance, assignment tests and the cluster analysis. Note Akwaizantar is not shown as no samples from Akwaizantar were included in these analyses.

GGNP central
GGNP east
GGNP southwest
Ngel Nyaki
GGNP boundary
Road

Spatial scale, linkage disequilibrium Hardy-Weinberg equilibrium

From the thirteen microsatellite loci originally selected, one failed to amplify any DNA, two were monomorphic and two had problems with stuttering or suspected artefacts that hindered the validation of the true genotypes (see Chapter two for details). These loci were dropped leaving eight loci in the final analyses. Linkage disequilibrium and Hardy-Weinberg equilibrium were assessed within sampling locations and at another spatial scale used in the following analyses. This latter spatial scale groups three pairs of sampling locations that are close to one another (approximately 10 km or less apart) into one location each. The rational for this was that very little is known about the ranging behaviour and territory size of these chimpanzees. The only published estimate of territory size in the Nigeria-Cameroon chimpanzee is that of a community in GGNP which is believed to be 26 km^2 (Sommer *et al.* 2004). If samples from locations that are in close proximity are from a single distinct community of chimpanzees then this would be a more appropriate scale to estimate genetic differentiation. The resulting effect is Ngel Nyaki forest reserve being treated as one region (instead of Ngel Nyaki and Danko), Mayo Fandam and Small Fandam becoming one region (GGNP southwest) and Gangirwal and Leinde Fadali becoming another (GGNP east, see Figure 3.1). In analyses using this spatial pattern the sampling location Ngiti is referred to as GGNP central to be more accordant with the names of the other regions and their reference to their respective locations in the park (see Figure 3.1).

Two loci (G00-228-893 and ATA28F03) are located on chromosome four and were checked for linkage disequilibrium using Arlequin ver 3.5 (Excoffier and Lischer 2010). When linkage was assessed within sampling locations, only in the samples from Mayo Fandam was there a significant pattern of linkage disequilibrium (X^2=15.55 df=8 P=0.049).

Table 3.1: Total number of samples collected and number of unique genotypes amplified for five or more loci and used in the analyses in this chapter, listed by forest names and the location in which that forest is situated. Forest fragment abbreviations are NN-Ngel Nyaki, DA-Danko, AK-Akwaizantar, MF-Mayo Fandam, SF-Small Fandam, NG-Ngiti, LF-Leinde Fadali, GA-Gangirwal.

	Ngel Nyaki forest reserve		Mambilla			Gashaka Gumti National Park		
Forest	NN	DA	AK	MF	SF	NG	LF	GA
Number of samples collected	16	13	6	8	8	11	10	10
Number of unique genotypes	4	5	0	5	5	7	5	6

When samples were grouped, the only region approaching significance for linkage disequilibrium was GGNP southwest (X^2=24.28 df=15 P=0.06), the region in which the samples at Mayo Fandam were included in.

A test for Hardy-Weinberg equilibrium (HWE) was conducted in GenAlex 6.5 (Peakall and Smouse 2006). Genotypes at locus ATA28F03 in the samples taken from Mayo Fandam when all sampling locations were considered separately, were the only samples to show a significant departure from HWE (see Table 3.2). All eight loci were used in all subsequent analyses as the pattern of linkage disequilibrium and the one deviation from

Table 3.2: Observed and expected (in italics) heterozygosity for each locus by sampling location and region. Bolded value represents a significant (P<0.05) departure from Hardy-Weinberg Equilibrium.

Locus	Ngel Nyaki	GGNP southwest	GGNP central	GGNP east
Mfd23	1	0.44	0.571	0.727
D16S265	*0.758*	*0.778*	*0.633*	*0.661*
G00-228-893	1	1	1	1
D4S1652	*0.750*	*0.805*	*0.681*	*0.826*
G00-218-317	0.714	0.857	1	0.818
D9S303	*0.694*	*0.735*	*0.625*	*0.785*
ATA28F03	0.667	0.750	0.5	1
D4S3248	*0.444*	*0.594*	*0.406*	*0.653*
GATA164B08P	0.857	1	0.833	0.889
D3S4545	*0.827*	*0.865*	*0.833*	*0.815*
GATA43C11	0.875	0.2	0.667	0.727
D7S1804	*0.695*	*0.32*	*0.681*	*0.698*
GATA14E09	0.625	0.556	0.143	0.545
D8S2324	*0.695*	*0.574*	*0.541*	*0.541*
ATA27A06P	0.889	0.9	0.714	0.727
D12S1042	*0.827*	*0.755*	*0.673*	*0.798*

Locus	Ngel Nyaki	Danko	Mayo Fandam	Small Fandam	Ngiti	Leinde Fadali	Gangirwal
Mfd23	1	1	0.5	0.4	0.571	0.6	0.833
D16S265	*0.719*	*0.688*	*0.75*	*0.58*	*0.633*	*0.54*	*0.722*
G00-228-893	1	1	1	1	1	1	1
D4S1652	*0.611*	*0.611*	*0.72*	*0.78*	*0.681*	*0.82*	*0.792*
G00-218-317	0.5	1	1	0.75	1	0.6	1
D9S303	*0.5*	*0.667*	*0.611*	*0.75*	*0.625*	*0.66*	*0.722*
ATA28F03	0.5	1	**1**	0.5	0.5	1	1
D4S3248	*0.375*	*0.5*	***0.5***	*0.625*	*0.406*	*0.625*	*0.625*
GATA164B08P	1	0.750	1	1	0.833	1	0.833
D3S4545	*0.722*	*0.750*	*0.78*	*0.84*	*0.833*	*0.778*	*0.792*
GATA43C11	0.75	1	0.2	0.2	0.667	0.6	0.833
D7S1804	*0.531*	*0.719*	*0.18*	*0.42*	*0.681*	*0.7*	*0.625*
GATA14E09	0.5	0.750	0.5	0.6	0.143	0.6	0.5
D8S2324	*0.594*	*0.719*	*0.563*	*0.58*	*0.541*	*0.48*	*0.569*
ATA27A06P	0.75	1	1	0.8	0.714	0.8	0.667
D12S1042	*0.656*	*0.8*	*0.8*	*0.64*	*0.673*	*0.8*	*0.681*

.

HWE were not consistent across all locations and were potentially a product of the small sample size.

Population structure and genetic diversity

Genetic diversity indices and an AMOVA were calculated in GenAlex 6.5. Population structure was also inferred using the program STRUCTURE ver. 2.3.4 (Pritchard *et al.* 2000). Genetic differentiation was assessed via AMOVA across a) all seven sampling locations, and b) when locations were grouped into four regions: Ngel Nyaki forest reserve, GGNP southwest, GGNP central and GGNP east. The first AMOVA assumed that the sampling locations reflect the spatial scale at which genetic differentiation occurs. Most samples were collected under nests in close proximity to one another in each forest suggesting that the chimpanzees sampled were from one social group. The samples from Ngel Nyaki were collected on two occasions. The forest at Ngel Nyaki is small (approximately seven km^2) in size and the existence of more than one community within the forest is unlikely. Two regions were also included in the first AMOVA. The first region comprised of Ngel Nyaki and Danko (i.e. Ngel Nyaki forest reserve) and the second included all locations in or on the edge of GGNP.

In the second AMOVA four populations were assumed, consolidating the samples collected in GGNP into three regions (see the section on 'spatial scale, linkage disequilibrium and Hardy-Weinberg equilibrium' for more details) and Ngel Nyaki forest reserve into one region. The necessary P value for significant pairwise F_{ST} values was adjusted for multiple testing using a modified false discovery rate method (Narum 2006) in both AMOVAs. The required P value for F_{ST} to be significantly different from zero in the AMOVA with seven sampling locations was $P<0.013$ and in the AMOVA with four regions was $P<0.02$.

Cluster analysis

The program STRUCTURE was used to perform model-based cluster analysis to determine how many populations exist. Whilst an AMOVA determines the level and pattern of genetic differentiation given pre-defined populations, cluster analysis determines the number and membership of populations given a genotypic data set. When the evidence for structure is weak the sensitivity of STRUCTURE is low. Evanno *et al.* (2005) developed a new statistic (ΔK) that is more sensitive at detecting the number of populations when dispersal is not homogeneous. However, this method has no power to distinguish between the number of groups (K) when $K = 1$ and $K = 2$. Another method is to include population information with the function LOCPRIOR which weights the prior probability distribution of

the number groups relative to sampling locations (Hubisz *et al.* 2009). This function has been suggested to improve performance in small data sets (Hubisz *et al.* 2009). Here I tested for the evidence of structure both with and without prior population information using the function LOCPRIOR because of the possibility that K = 1. When LOCPRIOR was used, samples were assigned to one of the four regions (Ngel Nyaki, GGNP southwest, GGNP central and GGNP east). In both instances the burn in length was set to 50,000 and the run length to 500,000 and a model with correlated allele frequencies was selected. Evidence of one to four groups was investigated.

Dispersal patterns

Patterns of dispersal were investigated using assignment tests to determine log likelihood values in the program Geneclass2 (Piry *et al.* 2004). Log likelihood values were calculated using the method of Paetkau *et al.* (1995) and the probability that an individual is a first generation migrant was estimated using a Monte Carlo resampling procedure (Paetkau *et al.* 2004). Calculating log likelihood values using this protocol has been shown to perform well under a range of conditions in particular when F_{ST} is low (Cornuet *et al.* 1999). The combination of this log likelihood protocol and the Monte Carlo resampling procedure have also been shown to generate the lowest type I error rate (wrongly detecting residents as immigrants) when identifying first generation migrants especially when populations sizes are small (Paetkau *et al*. 2004).

This protocol calculates the log likelihood that an individual's genotype belongs to each of the sampled locations given the allele frequencies calculated from the samples collected in that location. Likelihood values are calculated for each genotype at a particular locus as the square of allele frequencies for homozygote genotypes or twice the product of the allele frequencies for heterozygote genotypes. The resulting values are multiplied across all loci and log transformed to produce an overall log likelihood for each individual. Values are generated for each individual belonging to each location. Individuals are removed from the group of samples from where they were collected before estimating the allele frequencies used in calculating their log likelihood to that particular location, known as the 'leave one out' procedure (Efron 1983, Paetkau *et al.* 2004). To avoid zero likelihood values when an allele is missing from a population (therefore having a frequency of zero) the allelic frequency of missing alleles was set to 0.01. Assuming different values for missing alleles has been shown to have little effect on the overall power of the test so this was not explored further (Paetkau *et al.* 2004).

The probability that an individual is an immigrant is calculated by generating a distribution of likelihood values using Monte Carlo resampling methods. Simulated

individuals are generated from the allele frequencies for each location and likelihood values are calculated and ranked to generate a distribution from which critical values of a test statistic can be determined. Three different test statistics can be calculated (see Paetkau *et al.* 2004). The test statistic selected was L_H (the likelihood of obtaining an individual's genotype in the population it was sampled) which is the most appropriate test statistic to use when all potential source populations have not been sampled. Resampling occurs by selecting 'gametes' (not alleles) from the sample pool for each location. Resampling gametes has the advantage of preserving linkage disequilibrium that is present in first generation migrants (Paetkau *et al.* 2004). The number of simulated individuals was set to 1,000,000.

Patterns of log likelihood values were explored by plotting pairwise log likelihood values for the four regions (six pairwise comparisons) and between Ngel Nyaki and GGNP (i.e. migration into and out of the park). Tests for first generation migrants were similarly conducted at both these scales as these tests are based on allele frequencies which are sensitive to sample size so consolidating GGNP into one location may allow more accurate log likelihood values to be estimated for individuals originating from inside or outside the national park.

Results

Population structure

When the level and pattern of genetic differentiation was examined by treating all seven locations as separate populations, the global F_{ST} value for all locations was 0.065 (P = 0.001). Sub-populations within regions were significantly different from one another (F_{SR} = 0.042, P = 0.001) and regions (Ngel Nyaki forest reserve and GGNP) were significantly different from one another (F_{RT} = 0.024, P = 0.013). There was no significant differentiation (P<0.013 after correction for multiple testing to be equivalent to P<0.05) between all but one pair of sampling locations within GGNP (Table 3.3). Ngel Nyaki was significantly different from Gangirwal but F_{ST} values for all other locations except Leinde Fadali were approaching significance (Table 3.3). Danko was significantly different from three locations in GGNP. When the samples were grouped into four regions they all were significantly different from one another (P<0.02 after correction for multiple testing to be equivalent to P<0.05, see Table 3.4).

Table 3.3: Pairwise F_{ST} values for seven sampling locations. F_{ST} values are on the bottom diagonal and significant values (P<0.013 after correction for multiple testing) are bolded. Upper diagonal shows the original P values. Labels on the bottom row are abbreviations of the location names in the first column. The number in brackets is the number of samples/number of males in sample. Note the question mark next to Mayo Fandam because samples were not confirmed but suspected to be males (see Chapter two for more details).

Ngel Nyaki (4/0)	~	0.052	0.018	0.038	0.031	0.176	0.010
Danko (5/1)	0.055	~	0.020	0.011	0.013	0.035	0.001
Ngiti (7/6)	0.08	0.051	~	0.079	0.023	0.043	0.027
Small Fandam (5/0)	0.065	**0.074**	0.042	~	0.394	0.352	0.226
Mayo Fandam (5/2?)	0.071	**0.075**	0.066	0.003	~	0.051	0.009
Leinde Fadali (5/0)	0.028	0.052	0.052	0.011	0.053	~	0.192
Gangirwal (6/5)	**0.079**	**0.081**	0.046	0.019	**0.067**	0.020	~
	NN (5/0)	**DA (5/1)**	**NG (7/6)**	**SF (5/0)**	**MF (5/2?)**	**LF (5/0)**	**GA (6/5)**

Table 3.4: Pairwise F_{ST} values after locations were grouped. NNFR = Ngel Nyaki forest reserve, GGNPsw = Gashaka Gumti National Park southwest, GGNPe = Gashaka Gumti National Park east, GGNPc = Gashaka Gumti National Park central. Bolded values are significant (P<0.02 after correction for multiple testing). Upper diagonal shows the original P values. The number in brackets is the number of samples /number of males in sample. Note the question mark next to Mayo Fandam because samples were not confirmed but suspected to be males (see Chapter two for more details).

Ngel Nyaki FR (9/1)	~	0.012	0.002	0.002
GGNPc (7/6)	**0.048**	~	0.008	0.007
GGNPsw (10/2?)	**0.056**	**0.055**	~	0.013
GGNPe (11/5)	**0.042**	**0.047**	**0.032**	~
	NNFR (5/0)	**GGNPc (7/6)**	**GGNPsw (10/2?)**	**GGNPe (11/5)**

Genetic diversity

Genetic diversity indices are presented for Ngel Nyaki forest reserve and the three regions in GGNP. The range of the numbers of alleles per locus from these locations was 4.75 - 5.75 (Table 3.5). Mean observed heterozygosity was similar in all regions (range 0.679 - 0.828) and highest in Ngel Nyaki forest reserve. Mean expected heterozygosity was also similar across all regions (range 0.634 - 0.722) and highest in GGNP east. F_{IS} was not significantly different to zero for any region.

Cluster analysis

The most likely number of populations inferred from the genotype data was one when information on sampling locations was both excluded and included in the analysis (Figure 3.2).

Table 3.5: Genetic diversity indices for the four regions sampled. Italicised numbers represent standard deviations and standard error for the means or the range where indicated with a '*'. All F_{IS} values were not significant.

Location	Number of Samples	Mean number of alleles per locus	Mean observed heterozygosity	Mean expected heterozygosity	F_{IS}
Ngel Nyaki Forest Reserve	9	5.125	0.828 *0.051*	0.711 *0.043*	-0.181 *0.071*
GGNP central	7	4.750	0.679 *0.100*	0.634 *0.044*	-0.063 *0.144*
GGNP southwest	10	5.5	0.713 *0.102*	0.678 *0.062*	-0.023 *0.098*
GGNP east	11	5.75	0.804 *0.055*	0.722 *0.036*	-0.117 *0.066*
Mean (*Range)	**9.25 *9-11**	**5.28 *4.75-5.75**	**0.756** ***0.040***	**0.686** ***0.023***	**-0.096** ***0.049***

Dispersal – patterns of likelihood values

When the log likelihood values for pairwise regions within GGNP were compared (Figure 3.3 right side plots), samples did not score consistently higher for the location they were collected in (thus falling either side of the diagonal line). In contrast when samples collected in Ngel Nyaki forest reserve were plotted against samples from the three regions in GGNP, with the exception of the comparison with GGNP central, samples collected from Ngel Nyaki forest reserve tended to have higher log likelihood values for Ngel Nyaki forest reserve (therefore they are more likely to originate from Ngel Nyaki) and samples collected in the regions within GGNP tended to have higher log likelihoods for the location they were collected in (Figure 3.3, left side plots). The plot of Ngel Nyaki and GGNP central shows that one individual (sample 12) collected in Ngel Nyaki had a marginally higher likelihood value for GGNP central but two samples collected in GGNP central had considerably higher likelihood values for Ngel Nyaki than for GGNP central. When the origin of samples was designated either to be Ngel Nyaki forest reserve or just GGNP, log likelihood values were typically higher for the locations they were collected in (Figure 3.4). Two samples from Ngel Nyaki (12 and 118) had slightly higher log likelihood values for GGNP. Sample 76 from GGNP had a higher log likelihood value for Ngel Nyaki than it did for GGNP.

Dispersal – detection of first generation migrants

When four regions were analysed for first generation migrants, two samples are identified as migrants: sample 76 which was collected in GGNP central and but was identified as a first generation migrant from Ngel Nyaki (P=0.0002), and sample 96 collected in GGNP southwest which was identified as a migrant from GGNP east (P=0.005). The only

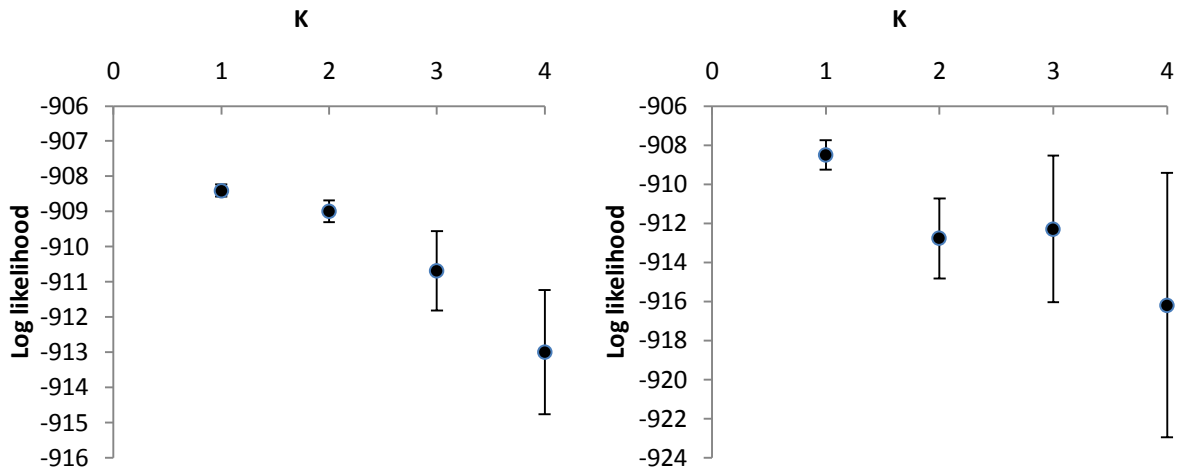

Figure 3.2: Log likelihood values for the number of groups (K) from one to four as inferred by STRUCTURE. The left plot shows log likelihood values when no prior population information was included in the model and right hand plot when it is included.

first generation migrant detected when examining Ngel Nyaki and GGNP as a whole was sample 76 (P=0.0006) collected in GGNP but indicated to have originated from Ngel Nyaki.

Discussion

The nature of chimpanzee communities is often reported as very dynamic (Goodall 1986, Mitani *et al*. 2002), sociable intergroup encounters and temporary associations between individuals do occur but males show hostility towards neighbouring communities and distinct territories exist (Boesch *et al*. 2008). Males of the species are known to be more gregarious than females (Mitani *et al*. 2000). When each sampling location was treated individually there was relatively little evidence of genetic differentiation, particularly between adjacent locations. For instance Mayo Fandam and Small Fandam had a pairwise F_{ST} of 0.003 (P=0.394, Table 3.3) and are located approximately 10 km apart. Likewise Gangirwal and Leinde Fadali are located at a similar distance from one another and had a pairwise F_{ST} of 0.02 (P=0.192, Table 3.3). When these two locations were grouped and the locations within Ngel Nyaki forest reserve were grouped all pairwise F_{ST} values were significant. The shortest distance between two locations within GGNP after grouping samples in this analysis was 30 km (GGNP central and GGNP east). The distance between Ngel Nyaki forest reserve and the next nearest sampling location (Mayo Fandam) is much shorter, 10-15 km, but they are divided by a road, a township and large tracts of agricultural land. These results are consistent with a previous estimate of territory size for one community in GGNP at 26 km^2 (Sommer *et al.* 2004) and suggest that the 'regions' analysed in this study may represent a spatial scale that encompasses the territories of distinct communities within the park.

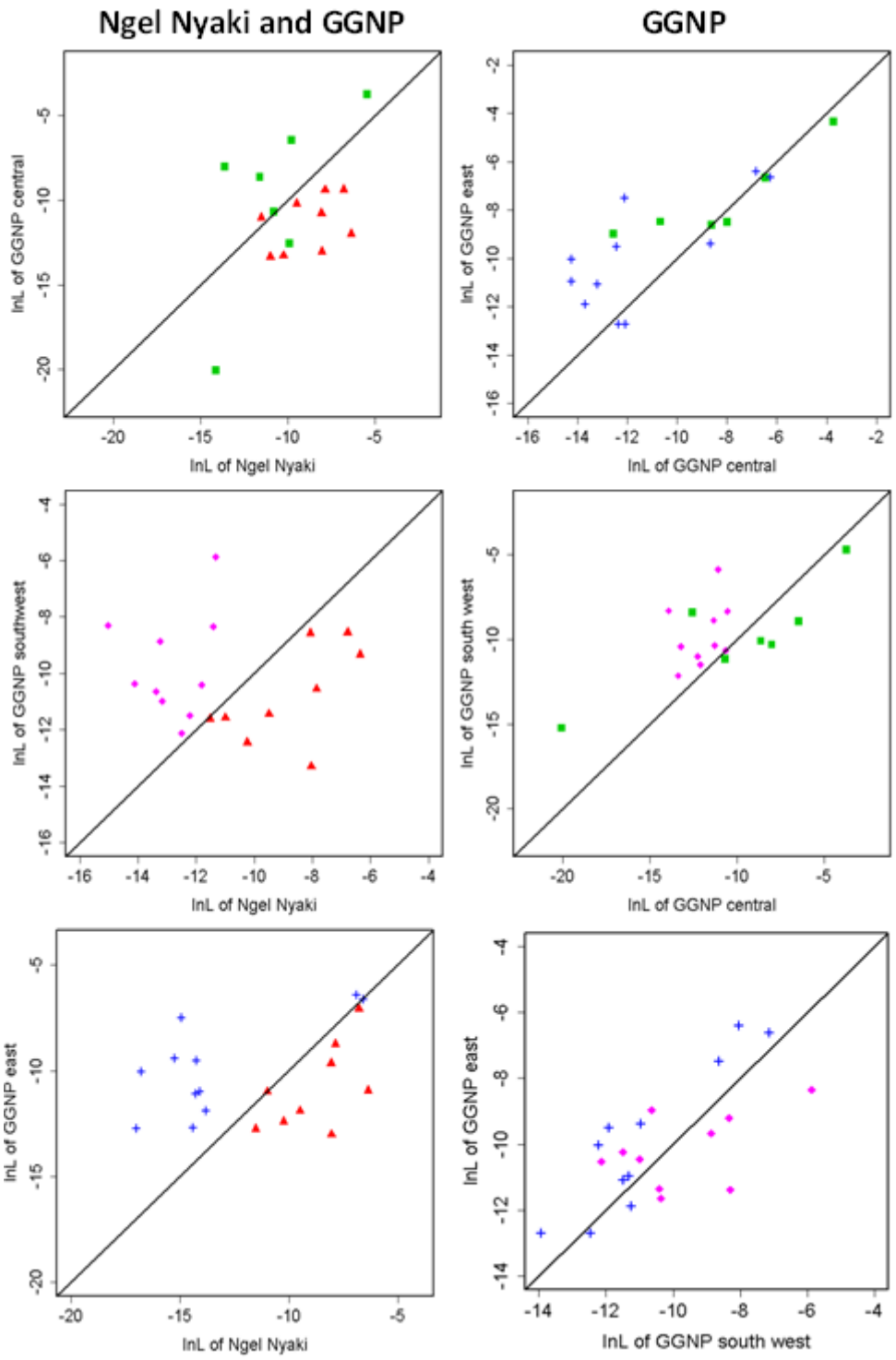

Figure 3.3: Pairwise log likelihood values for assignment test. Samples collected from each location are represented by different symbols (red triangle= Ngel Nyaki, blue cross=GGNP east, purple dot=GGNP southwest, green square=GGNP central. Plots on the left show the comparisons between Ngel Nyaki and all locations within the GGNP. Plots on the right show comparisons between locations within GGNP. Diagonal lines represent the point where likelihood values are equal for each population. Symbols to the left of the line are samples with likelihood values suggesting they are more likely to originate from the location on the y axis, and symbols to the right have likelihood values suggesting they are more likely to originate from the location on the x axis.

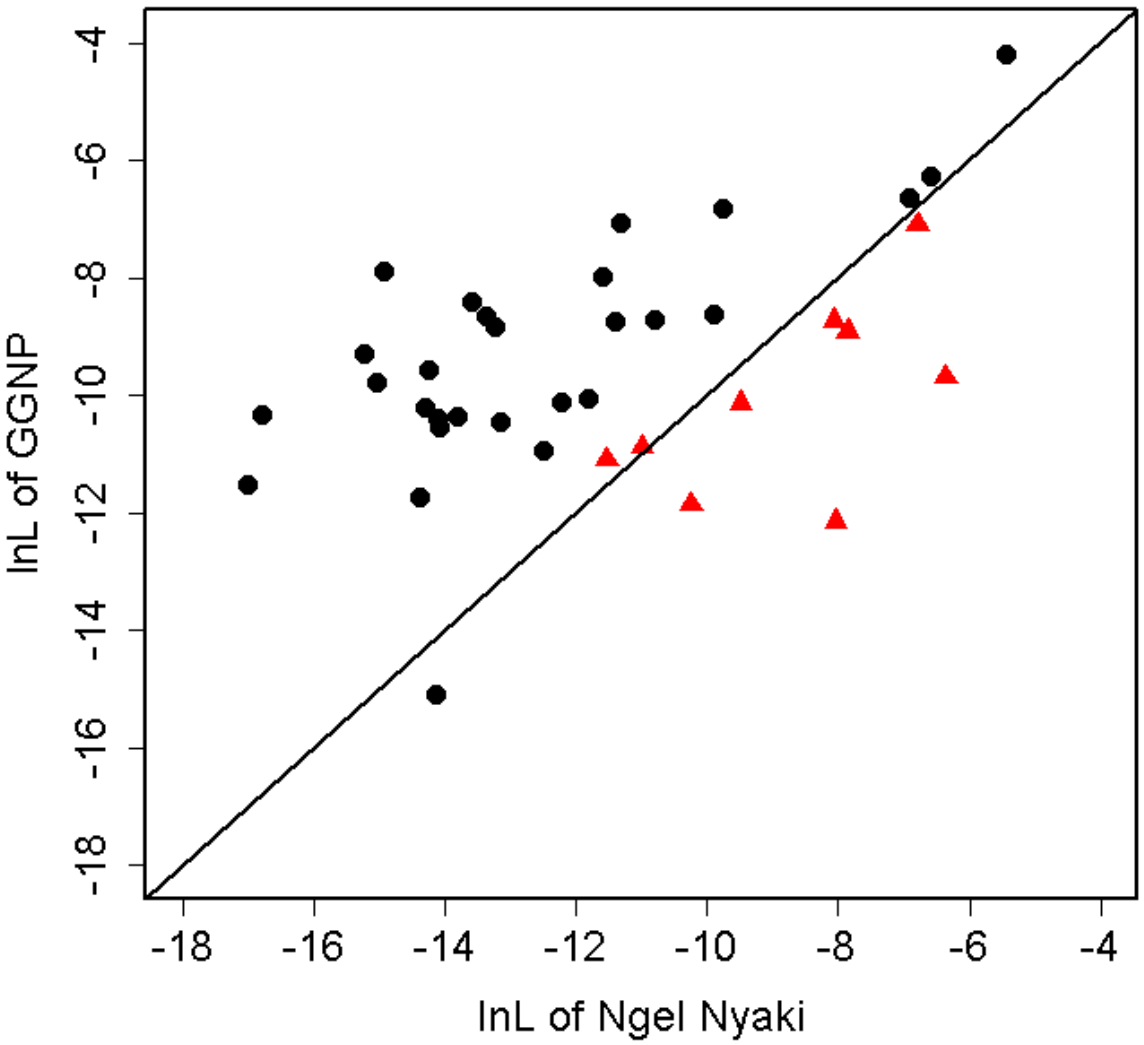

Figure 3.4: Log likelihood values for all samples belonging to either Ngel Nyaki or GGNP. Samples collected in Ngel Nyaki are represented by red triangles and samples collected in GGNP are represented by black circles. Diagonal line represents point where likelihood values for samples to belonging to either population are equal. Points on the left of the line are more likely to originate from GGNP and points on the right of the line are more likely to originate from Ngel Nyaki.

An interesting point to note is that when the analysis was conducted on the four regions, all of the regions contained males or suspected males (Table 3.3 and 3.4). Males are reported as being philopatric in other sub-species of chimpanzee (Mitani *et al*. 2002) and this could be driving the significant differentiation.

Is Ngel Nyaki reserve isolated from GGNP? There was a significant differentiation between Ngel Nyaki forest reserve and all three of the regions within GGNP. F_{ST} was not, however, much greater than it was between pairs of regions within GGNP. The assignment tests did show a modest tendency for samples collected from Ngel Nyaki forest reserve to have higher log likelihood values for Ngel Nyaki and likewise for samples from the regions within GGNP. This pattern was not apparent between pairwise comparisons of regions

within GGNP. This would indicate that allele frequencies are more similar between any two regions in GGNP than they are between Ngel Nyaki forest reserve and any region in GGNP. Therefore gene-flow is potentially less restricted between sites in the national park than it is between Ngel Nyaki forest reserve and GGNP.

Yet despite this fact, one of the two migrants detected in GGNP was estimated to originate from Ngel Nyaki forest reserve. Information on the development of infrastructure in Nigeria is notoriously difficult to obtain. The road that runs between Ngel Nyaki forest reserve and GGNP was probably paved during the 1970s or 1980s and human presence in the area would have increased steadily since then (Hazel Chapman, personal communication). Chimpanzees are known to live longer than 30 years in the wild (Goodall 1986) and this individual may have transferred between Ngel Nyaki forest reserve and GGNP when human population density in the region between these locations was lower than it is now. It is also possible that this individual originates from an unsampled population whose allele frequencies are similar to the community at Ngel Nyaki forest reserve. Another explanation is that the sample size from Ngel Nyaki forest reserve was small (nine) and so the allele frequencies might not be accurately represented there hence the log likelihood value may be misrepresentative of the true population of origin. Although a previous population size estimate calculated 12 individuals in Ngel Nyaki forest reserve (Beck and Chapman 2008) which would suggest that this was probably not the case. Identifying this individual as a migrant is intriguing as the sample is believed to be a male and is contrary to expectations of female biased dispersal (Langergraber et al. 2007) in chimpanzees (see Chapter four for more detail).

Due to the long generation time of chimpanzees and the estimated date of the road being paved the pattern of log likelihood values may be indicative of early isolation of Ngel Nyaki forest reserve from GGNP. If it has become isolated only recently creating weak population structure this would also explain the inability of the program STRUCTURE to detect differentiation as it has been shown to have low sensitivity when population structure is weak (Evanno *et al*. 2005).

The lack of significant F_{IS} values in any population indicates that inbreeding is not apparent. The community at Ngel Nyaki forest reserve actually had the highest observed heterozygosity of all locations. The population at Ngel Nyaki forest reserve might not be experiencing a reduction in reproductive fitness at present but the fact that only one male was sampled raises concerns about the immediate future of the population.

Chapter 4 Sex Biased Dispersal

Introduction

A broad definition of dispersal is the movement of an individual from one location to another resulting in gene-flow (Greenwood 1980, Starrfelt and Kokko 2012). Moving through an unfamiliar environment is inherently risky; predation, energetic costs and the inability to locate resources can increase the chance of mortality. Hence the advantages of dispersal must outweigh the costs for dispersal to have evolved. For example, not dispersing may increase the costs on residents through density dependent processes whereas dispersing can benefit individuals by alleviating competition between kin (Hamilton and May 1977, Lambin *et al.* 2001). In this latter context parent organisms can also increase their inclusive fitness by encouraging offspring to leave. In heterogeneous environments the increased risk of local extinction is expected to select for dispersal (Gandon and Michalakis 2001). Another explanation is that organisms disperse to avoid inbreeding, although for dispersal to evolve in response to inbreeding only one sex needs to disperse (Starrfelt and Kokko 2012).

Sex biased dispersal

In the animal kingdom the tendency for the dispersal characteristics of one sex in a species to differ from the other sex occurs frequently (Lawson Handley and Perrin 2007). Possibly the most established contrast in many species is the differential rate of dispersal between the sexes, but the timing of dispersal and the distance dispersed may also vary (Fontanillas *et al.* 2004, Gauffre *et al.* 2009). Variation between the sexes in these dispersal characteristics can occur at two distinct stages, either when an organism leaves its natal site to its place of first reproduction (natal dispersal) and/or breeding dispersal when an organism moves to successive breeding sites (Greenwood 1980).

Patterns of sex biased dispersal are apparent at both broad and fine taxonomic scales. For instance, mammals generally tend to display male biased dispersal and birds generally exhibit female biased dispersal (Greenwood 1980, Dobson 1982). For example within new world primates, capuchin monkeys (family *Cebidae*) conform to the pattern of mammalian male bias (Jack and Fedigan 2004a, 2004b) in contrast to members of the family *Atelidae* (spider, squirrel and howler monkeys) in which it is the females who are

frequently the dispersing sex (Nishimura 2003). These patterns are often modelled as a consequence of the interaction between inbreeding avoidance and the asymmetric costs of different forms of competition. More recently theories have emerged indicating the role of cooperative behaviour in the evolution of sex bias dispersal (Greenwood 1980, Dobson 1982, Lawson Handley and Perrin 2007).

Greenwood (1980) hypothesized that the pattern in sex bias dispersal was highly correlated with the type of mating system, relating to different forms of competition. For example the strong resource competition frequently observed in birds selects for male philopatry. Males defend territories and benefit from philopatry through familiarity with the area. Females are forced to disperse to avoid inbreeding and select mates potentially on the basis of territory quality. In contrast female defence polygyny, where males defend harems of females, selects for male dispersal as males are limited by their ability to mate locally. Strong reproductive competition between local males selects for male biased dispersal (Greenwood 1980, Dobson 1982). This hypothesis has since been supported by considerable empirical evidence. A review of mammalian dispersal showed a link between local mate competition and male dispersal in eight out of twenty species exhibiting a male bias (Lawson Handley and Perrin 2007). Moreover local resource competition or resource defence was attributed to female bias dispersal in fourteen out of sixteen species showing female biased dispersal. Likewise female bias appears to be predominant in avian taxa, although the occurrence of male bias is not as rare as once thought (Clarke *et al.* 1997).

The number of exceptions to the general rule of mammalian and avian dispersal has been steadily accumulating (Clarke *et al.* 1997, Coulon *et al.* 2006). Conditions that favour changes to the predicted pattern of bias arise when alternative selective pressures exert a greater effect than that of competition. For example, if male tenure of harems in polygynous species exceeds that of age to first conception in females, the probability of inbreeding between father-daughter pairs increases. Since a female's investment in offspring is typically greater than male investment, the cost of inbreeding is greater for the female and selects for female dispersal. This pattern has been noted in several polygynous mammals (Clutton-Brock 1989, Nagy *et al.* 2007), although this greater cost can be countered by females if they engage in mate choice (Perrin and Goudet 2001). Actively avoiding inbreeding by selecting unfamiliar males should promote male biased dispersal in polygynous systems (Lehman and Perrin 2003). Packer (1979) noted that female olive baboons (*Papio anubis*) do indeed show preference for immigrating males. Correspondingly males also preferred 'foreign' females during intergroup encounters.

Another emerging hypothesis is the influence of kin cooperation on dispersal patterns. Perrin and Lehman (2001) provided a theoretical basis on which the ability to recognise kin in more cognitively advanced species could emphasize the benefits of philopatry and lead to the development of sociality among kin. However Schultz *et al.* (2011) modelled the evolutionary relationships between extant primates and offered an opposing view. In this model, sociality evolved as a response to predation and sex biased dispersal arose after the evolution of group structures. However empirical evidence for the relative importance of kin cooperation is scarce. Le Galliard *et al.* (2006) who demonstrated that female microtine rodents cluster together speculated this was to assist in protection from infanticidal males. Furthermore dispersal characteristics may be plastic; evidence suggests that rates of dispersal can be density dependent so that the role of kin cooperation becomes more prominent in highly saturated environments (Lambin *et al.* 2001).

Sex biased dispersal in apes

Within non-human hominid lineages dispersal patterns are varied. A study on the relatedness of Bornean orang-utans (*Pongo pygmaeus*) at a fine scale (<10 km^2) showed that there was no obvious difference between residents of either sex (Goossens *et al.* 2006). Samples taken from across Borneo and Sumatra however have indicated that dispersal is male biased (Nater *et al.* 2011, Nietlisbach *et al.* 2012). In Western lowland gorillas (*Gorilla gorilla gorilla*) both sexes are obligate natal dispersers but dispersal distance might vary significantly (Douadi *et al.* 2007). For males uniparentally inherited markers revealed no evidence of population structure across a 6000 km^2 region. Females in contrast may be far more restricted in their movement as indicated by high levels of among group variation in mitochondrial DNA (Douadi *et al.* 2007). Bradley *et al.* (2007) found that 40% of female gorillas have a relative in the same group. It has been proposed that females disperse to the adjacent breeding group in a 'stepping stone' model (Douadi *et al.* 2007).

It is the females in both chimpanzee species (*Pan troglodytes* and *P. paniscus)* that are believed to be the predominant dispersers (Mitani *et al.* 2002, Eriksson *et al.* 2006). Variation in Y-chromosome markers has been shown to be highly community specific in bonobos whereas mitochondrial DNA variation is more ubiquitous (Eriksson *et al.* 2006). Observations on eastern chimpanzees (*P. t. schweinfurthii*) have highlighted that it is females that leave the natal site more frequently (Goodall 1986, Nishida 1990). This has been backed up with genetic data, much like the bonobos, such that Y-chromosome markers are often population specific unlike mitochondrial DNA markers (Langergraber *et al.* 2007). In a small community of western chimpanzees (*P. t.* verus) at Bossou in Guinea, both males and females have been reported to emigrate. Emigrants have not been reported in adjacent

communities. Neither has female immigration into the community ever been recorded since observations began in 1976 (Sugiyama 2004, Shimada *et al.* 2008).

A diverse number of potential evolutionary pressures exist that could influence patterns of dispersal in chimpanzees. The mating system of the common chimpanzee is extremely flexible. Both sexes are promiscuous but long term associations and pair bonds have been observed (Stumpf and Boesch 2005, Langergraber *et al.* 2013). Levels of promiscuity may potentially vary with fertility and females can switch from a 'many male' strategy to a 'best male' strategy at will (Matsumoto-Oda 1999, Stumpf and Boesch 2005). Males engage in aggressive coercion, opportunistic mating, possessive short term relationships and coalitionary mate guarding (Tutin 1979, Watts 1998, Muller *et al.* 2007). Furthermore males are generally more gregarious than females (Goodall 1986) and intragroup relatedness is believed to be higher between males than females (Morin *et al.* 1994). These observations have led to the supposition that kin selection may be important in the formation of male groups (Morin *et al.* 1994, Mitani *et al.* 2002), although some have argued that opportunistic 'political' or social motives may also drive male social interactions (de Waal 1984, Mitani 2006).

While female biased dispersal has been noted in other sub-species of the common chimpanzee it has not been tested for in the Nigeria-Cameroon chimpanzee (*P. t. ellioti*). In this chapter I investigated sex biased dispersal in the Nigeria-Cameroon chimpanzee between Ngel Nyaki forest reserve and Gashaka Gumti National Park (GGNP) and within GGNP alone. GGNP is home to possibly the largest remaining population of the Nigeria-Cameroon chimpanzees estimated to number between 1000 and 1500 (Morgan *et al.* 2011). In Ngel Nyaki forest reserve there may be approximately 12 chimpanzees residing in the forest (Beck and Chapman 2008).

Methods

I collected 85 faecal samples from eight locations in Taraba State, Nigeria. Three of the locations are within GGNP (Ngiti, Leinde Fadali and Gangirwal), two are just outside the southern boundary of the park (Mayo Fandam and Small Fandam) and two are located inside Ngel Nyaki forest reserve (Ngel Nyaki and Danko) approximately 10-15 km south of GGNP (see Figure 4.1). The proximity of Mayo Fandam and Small Fandam to GGNP and the fact that the land between these locations is sparsely inhabited suggests that movement by chimpanzees between them is not restricted, hence they are treated as part of GGNP in the following analyses. Samples were also collected from a location approximately 30 km

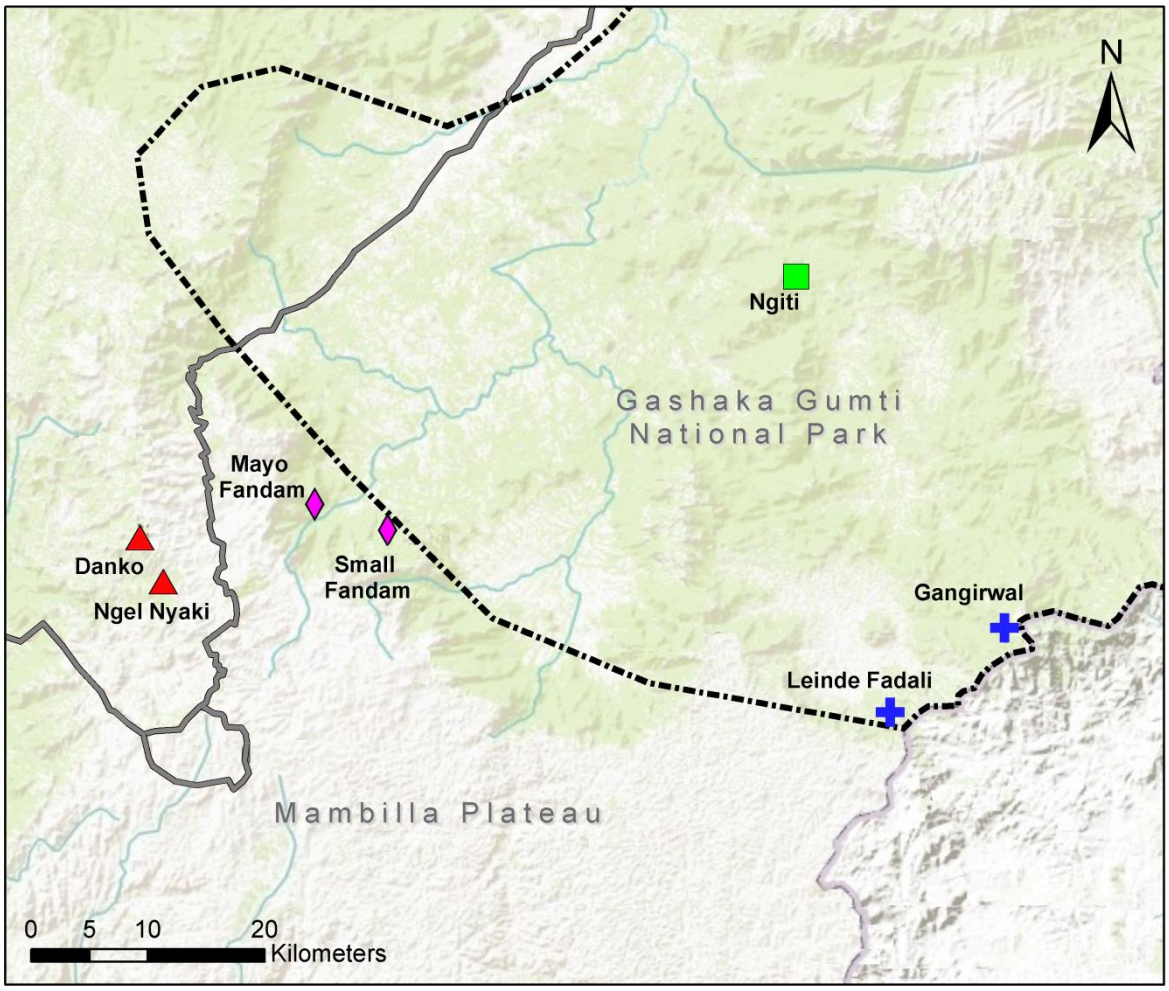

Figure 4.1: Map of the sampling locations in this study, symbolized according to the population structure assumed in the F_{ST} and assignment tests (see text for more detail).

southwest of Ngel Nyaki forest reserve (Akwaizantar) but these failed to amplify sufficient DNA to be included in the following tests.

Genotypes for these samples were determined using eight microsatellite loci (see Chapter two for more detail). The sex of the chimpanzees was ascertained using the sex specific amelogenin primer (Bradley *et al.* 2001). The amelogenin primer amplifies a 106 bp fragment on the X chromosome and a 112 bp fragment on the Y chromosome. Thus if the sample comes from a female the genotype will be a homozygote for the 106 bp fragment. If the sample comes from a male then the genotype will be a heterozygote for the 106 bp and

112 bp fragments. Genotypes and sexes used in these analyses were confirmed following protocols outlined in Chapter two. Thirty five unique genotypes whose sexes were confirmed were used in the following tests.

Sex biased dispersal may arise as a simple function of sex ratios; therefore all sex ratios are reported in the results. Another caveat is the effect of isolated populations from which dispersal may not be possible. If the two forest fragments in Ngel Nyaki forest reserve are isolated (see Chapter three for more detail) this may confound results. Hence each test was conducted both including and excluding the samples collected within this reserve. Four tests for sex biased dispersal were employed. Since no a priori knowledge of the Nigeria-Cameroon chimpanzee's dispersal characteristics are known the first two tests were chosen based on the power to detect a sex bias under different conditions.

Sex specific F_{ST}

The first test compares sex specific F_{ST} values. F_{ST} measures the mean reduction in heterozygosity in a sub-population compared to the total population. Since F_{ST} is a measure of genetic differentiation among sub-populations the more philopatric sex should exhibit higher values of F_{ST}. This test has been shown to be effective when the bias is relatively weak and dispersal is moderately frequent (Goudet *et al.* 2002). If dispersal is very frequent then populations become homogeneous and this method loses power. If dispersal is infrequent, however, immigrants represent a small proportion of the population and may go unsampled (Goudet *et al.* 2002). A similar test is possible with sex specific F_{IS} values as F_{IS} should be greater in the dispersing sex due to the Wahlund effect (pooling across immigrants and residents). This method has been shown to have less power when compared with F_{ST} and assignment tests and therefore it was not included in this analysis (Goudet *et al.* 2002).

This test requires a pre-determined populations to be selected between which F_{ST} is measured. Four populations were assumed to exist: Ngel Nyaki forest reserve, GGNP southwest, GGNP central and GGNP east. All of these locations had significant pairwise F_{ST} values as determined by an analysis of molecular variance (see Figure 4.1 and Chapter three for more detail). Furthermore the minimum distance between the locations were samples were collected in these regions is approximately 30 km. Sommer *et al.* (2004) estimated that the home range of a community in GGNP was approximately 26 km^2. Consolidating the samples into these four populations may reflect the social structure of the chimpanzees in GGNP. Global F_{ST} values, calculated using GenAlex 6.5 (Peakall and Smouse, 2006), for all populations across all loci were compared using a nonparametric

Wilcoxon t-test in R ver 3.0.1 (R core team 2013) because the distribution of F_{ST} values was non-normal and the variances were not equal when samples from the whole study area were included (Zar 1999).

Assignment test

When the rate of dispersal is very low comparing the variance of corrected assignment indices has been shown to be more sensitive than the F_{ST} test (Goudet *et al.* 2002). Assignment indices (AI) for each individual were calculated as the product of the squared allele frequencies for homozygous loci and twice the product of the allele frequencies for heterozygous loci given the allelic frequencies for their putative populations. The mean AI for each sub-population was subtracted from each individual's AI within that sub-population after log transformation. The remainder was the corrected assignment index (AI_C). Distributions of AI_C values should be bimodal if one sex disperses more frequently. The philopatric sex should show a skew towards positive AI_C values and the dispersing sex towards negative values (Favre *et al.* 1997). The variances of the dispersing sex should be greater than that of the philopatric sex because populations will contain residents and migrants from the dispersing sex, whereas there should be more residents of the predominantly philopatric sex (Goudet *et al.* 2002). Assignment indices were calculated in GenAlex 6.5 based on the same four populations used in the previous test (Ngel Nyaki, GGNP central, GGNP southwest and GGNP east). When the allele frequencies were estimated to calculate the assignment index for an individual to the population that the individual was sampled in, the individual's genotype was included in the calculation of allele frequencies by using the 'as is' function. The variance of sex specific AI_C values was compared using an F test in R ver 3.0.1.

Mantel test

As the calculation of both F_{ST} and assignment indices are based upon allelic frequencies they are sensitive to sample size. Both methods also require the assumption of a population structure which has to be inferred if it is not known. Due to this a Mantel test was used to assess the correlation between pairwise relatedness and geographical distance for males and for females. Two Mantel tests were conducted, one including the samples collected in Ngel Nyaki forest reserve and one excluding them. Pairwise relatedness was calculated using Queller and Goodnight's (1989) measure because it does not have a downward bias when applied to small sample sizes. Pairwise geographical distances between sample locations were calculated using GPS data collected in the field.

Pairwise relatedness

Recent studies have highlighted the low power associated with Mantel tests thus a fourth test was employed (Guillot and Rousset 2013). This test examines the relationship between mean ranked pairwise relatedness and mean geographical distance (Knight *et al.* 1999). Regression on pairwise relatedness raises the problem of pseudo-replication because for *n* individuals there will be *n(n-1)/2* pairwise relatedness values. To account for this, pairwise relatedness values for individual *i* and all other individuals can be calculated and then ranked according to relatedness. This was done for all individuals. The mean relatedness and corresponding mean distance for the highest rank across all individuals was determined. Then the mean relatedness and mean distance for the second highest rank across all individuals was calculated and so forth for all ranks (Knight *et al.* 1999). This results in *n-1* data points as there is no pairwise value between an individual and itself. This was done separately for each sex. The mean ranked relatedness was then regressed against the corresponding mean distances to estimate the relationship between them for each sex. Pairwise relatedness was calculated using Queller and Goodnight's (1989) measure of relatedness. This process was done twice, once for males and females in all locations and once for males and females in GGNP alone.

Results

Sex ratios

Overall the sex ratio was 37:63 (males:females). In the GGNP however the observed ratio was more even at 46:54 (males:females). In Ngel Nyaki forest reserve the sample was very female biased at 11:89 (males:females), see Table 4.1.

Table 4.1: The number of each sex sampled in each location, GGNP is further divided into the three regions used in the F_{ST} and assignment tests applied in this chapter.

Location	Males	Females
Ngel Nyaki Forest Reserve	1	8
GGNP	12	14
GGNP south west	0	8
GGNP central	6	1
GGNP east	6	5
Total	**13**	**22**

Sex specific F_{ST}

The results of the F_{ST} test show that there was a significant difference between F_{ST} values for males and females in GGNP ($W = 1$, $P < 0.001$, Table 4.2). There was no significant difference in global F_{ST} values when Ngel Nyaki forest reserve was included in the analysis ($W = 24$, $P = 0.44$, Table 4.2).

Assignment test

Variance in the AI_C scores for males and females in GGNP were not significantly different from one another ($F_{11,13}$ = 2.0653, P = 0.214, Table 4.3). Neither were they when Ngel Nyaki was included in the analysis ($F_{12,21}$ = 2.074, P = 0.078, Table 4.3). The distributions of the AI_C scores are similar (Figure 4.2). The mode of the AI_C values for females is less than males both when Ngel Nyaki forest reserve is included and excluded (Figure 4.2).

Mantel test

There was a significant correlation between geographical distance and pairwise relatedness for males when Ngel Nyaki forest reserve was included (r^2 = 0.0386, P = 0.039, Figure 4.3) and excluded (r^2 = 0.04, P = 0.04, Figure 4.4) from the analysis. The correlation between geographical distance and pairwise relatedness was not significant for females when Ngel Nyaki forest reserve was included (r^2 = 0.0092, P = 0.093, Figure 4.3) and excluded (r^2 = 0.0031, P = 0.308, Figure 4.4).

Table 4.2: Sex specific F_{ST} values for all loci calculated using samples from all locations and samples from GGNP only. The number of samples collected for each sex in the locations included in that analysis are given below their respective genders. Wilcoxon t-test results for the comparison between F_{ST} values for males and females are shown at the bottom.

	All sampling locations		**GGNP only**	
Locus	Females 22	Males 13	Females 14	Males 12
Mfd23 *D16S265*	0.248	0.497	0.272	0.065
G00-228-893 *D4S1652*	0.124	0.179	0.115	0.086
G00-218-317 *D9S303*	0.380	0.169	0.462	0.081
ATA28F03 *D4S3248*	0.463	0.573	0.496	0.050
GATA164B08P *D3S4545*	0.262	0.198	0.268	0.059
GATA43C11 *D7S1804*	0.150	0.077	0.187	0.030
GATA14E09 *D8S2324*	0.260	0.119	0.300	0.141
ATA27A06P *D12S1042*	0.270	0.235	0.308	0.043
Mean	0.270	0.256	0.301	0.069
Standard Error	0.039	0.064	0.045	0.012
Variance	0.012	0.032	0.016	0.001
Wilcoxon t-test	**W = 24 P = 0.44**		**W = 1 P <0.001**	

Table 4.3: F test results and AI_C scores for males and females in all sampling locations and when the data is restricted to GGNP alone.

	All sampling locations		**GGNP**	
Sex	Females	Males	Females	Males
Sample Size	22	13	14	12
AI_C scores	-0.152	-0.929	0.293	1.753
	1.562	1.753	0.009	-1.063
	-1.611	-1.063	0.732	2.952
	-0.657	2.952	-0.130	-0.433
	-0.902	-0.433	-0.121	0.418
	1.640	0.418	-0.364	-3.920
	0.334	-3.920	-0.586	-0.882
	0.714	-0.882	-0.197	-1.156
	0.293	-1.156	0.656	0.054
	0.009	0.054	-1.573	1.440
	0.732	1.440	2.154	0.238
	-0.130	0.238	2.398	0.071
	-0.121	0.071	-0.826	
	-0.364		-1.919	
	-0.586			
	-0.197			
	0.656			
	-1.573			
	2.154			
	2.398			
	-0.826			
	-1.919			
Variance	1.349	2.800	1.447	2.98
F test	**$F_{12,21}$ = 2.074 P = 0.1382**		**$F_{11,13}$ = 2.0653 P = 0.214**	

Pairwise relatedness

The regression on the mean ranked pairwise relatedness and corresponding mean geographical distance was significant for males when Ngel Nyaki forest reserve was included (r^2 = 0.42 $t_{1,10}$ = -2.741 P = 0.0208, Figure 4.5) and excluded (r^2 = 0.46 $t_{1,9}$ = -2.775 P = 0.0216, Figure 4.6). Mean distance had a negative relationship with mean relatedness. The regression was not significant for females when Ngel Nyaki forest reserve was included (r^2 = 0.06 $t_{1,20}$=-1.125 P=0.275, Figure 4.5) or excluded (r^2 = 0.002 $t_{1,11}$=-0.155 P=0.880, Figure 4.6).

Discussion

The four tests of sex biased dispersal conducted in this chapter produced equivocal results. If the Nigeria-Cameroon chimpanzee is similar to other chimpanzee sub-species in their dispersal behaviour then we would expect to find evidence of female biased dispersal. For the first test, comparisons of sex specific F_{ST} values, we would therefore expect higher

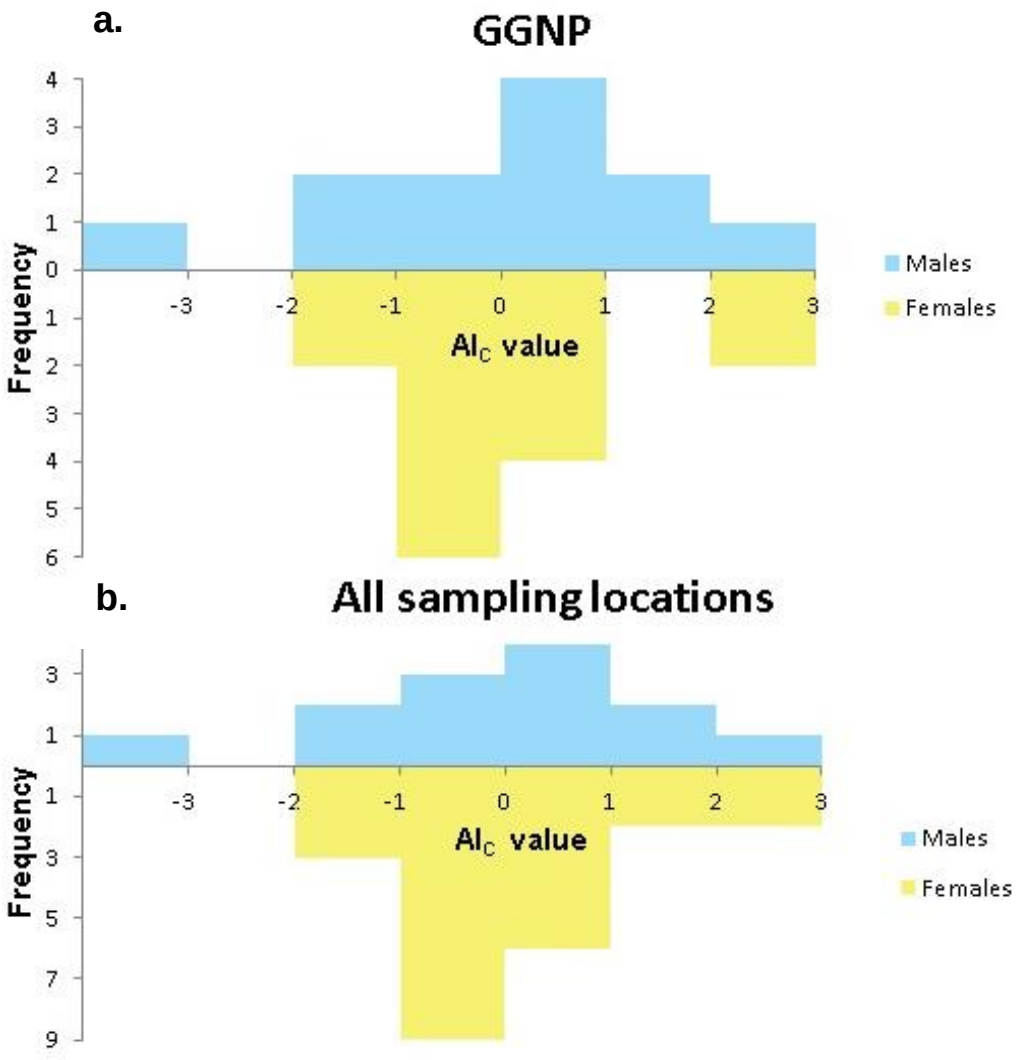

Figure 4.2: Distribution of AI_C values for a) GGNP and b) all sampling locations.

F_{ST} values for males than for females. However this test showed a significant difference in the global F_{ST} values between males and females only when the samples from GGNP alone were included in the test. Contrary to the results expected under female biased dispersal, F_{ST} was higher for females than it was for males implying less dispersal by females. This result however, was only apparent when a particular female was included in the analysis. This was the only female to be sampled in the GGNP central region, the northern most region in the whole study area. This individual was homozygous for four of the six loci that it was successfully typed for. The mean heterozygosity calculated for all sub-populations reduced by 0.19 when this individual was included in the analysis which resulted in a higher value of F_{ST}. When this individual was excluded from the analysis the mean F_{ST} value across all loci for females dropped from 0.301 to 0.045 which was lower than the value of F_{ST} for males (0.069) and conducive with patterns of female biased dispersal. A Wilcoxon t-test between F_{ST} values when this individual was removed showed no significant difference

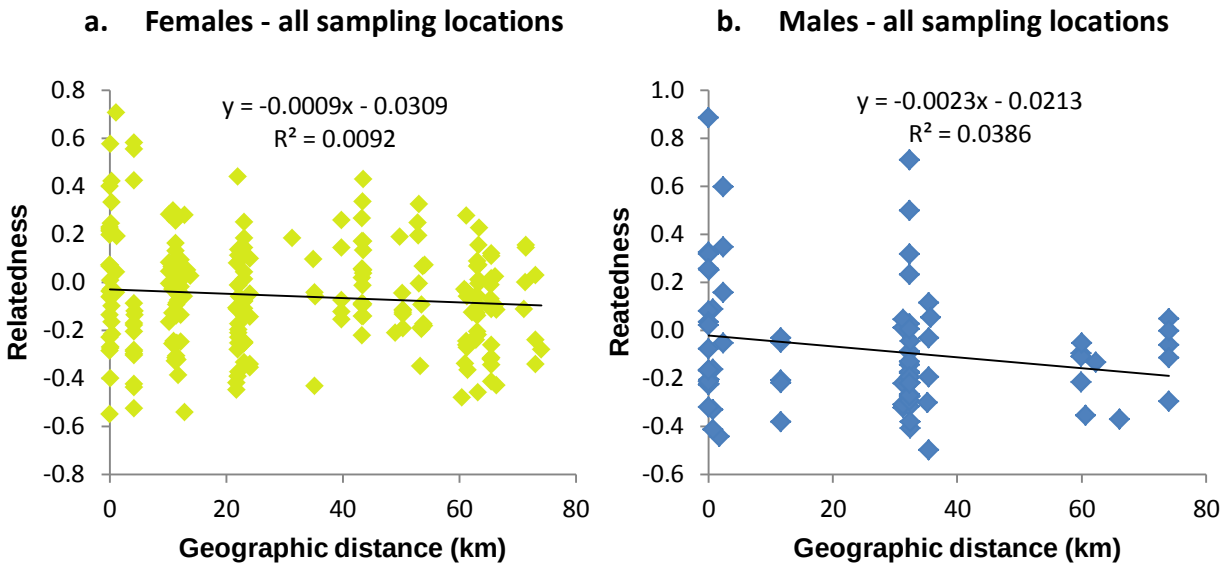

Figure 4.3: The correlation between geographic distance and pairwise relatedness as determined using a Mantel test for a) females and b) males from all sampling locations are included in the test.

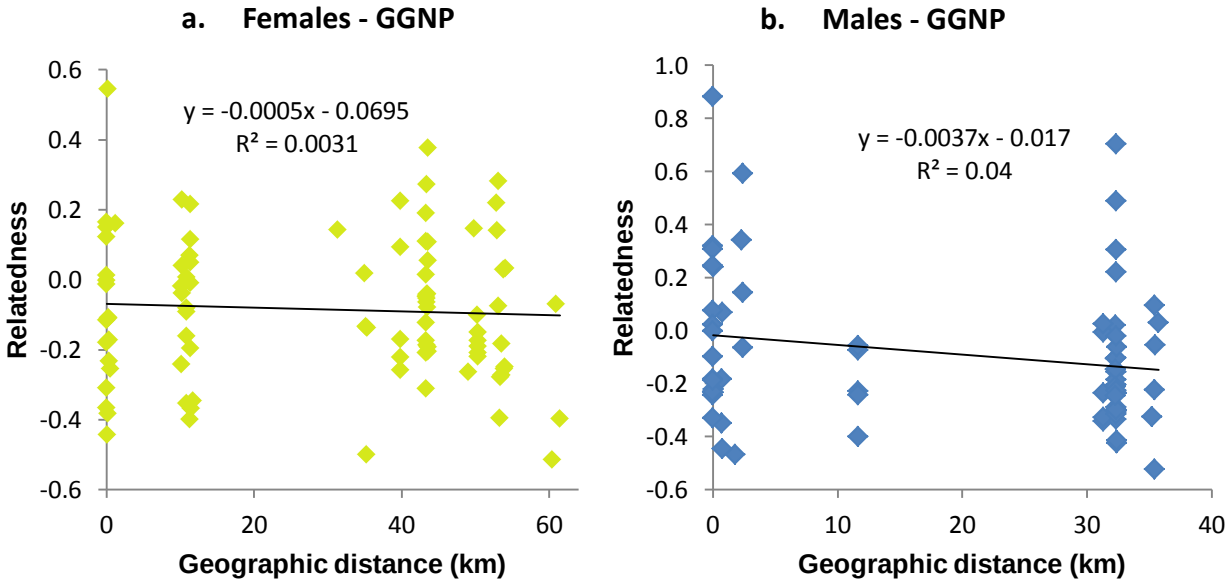

Figure 4.4: The correlation between geographic distance and pairwise relatedness as determined using a Mantel test for a) females and b) males when only individuals from GGNP and the forest fragments located just outside the park borders are included.

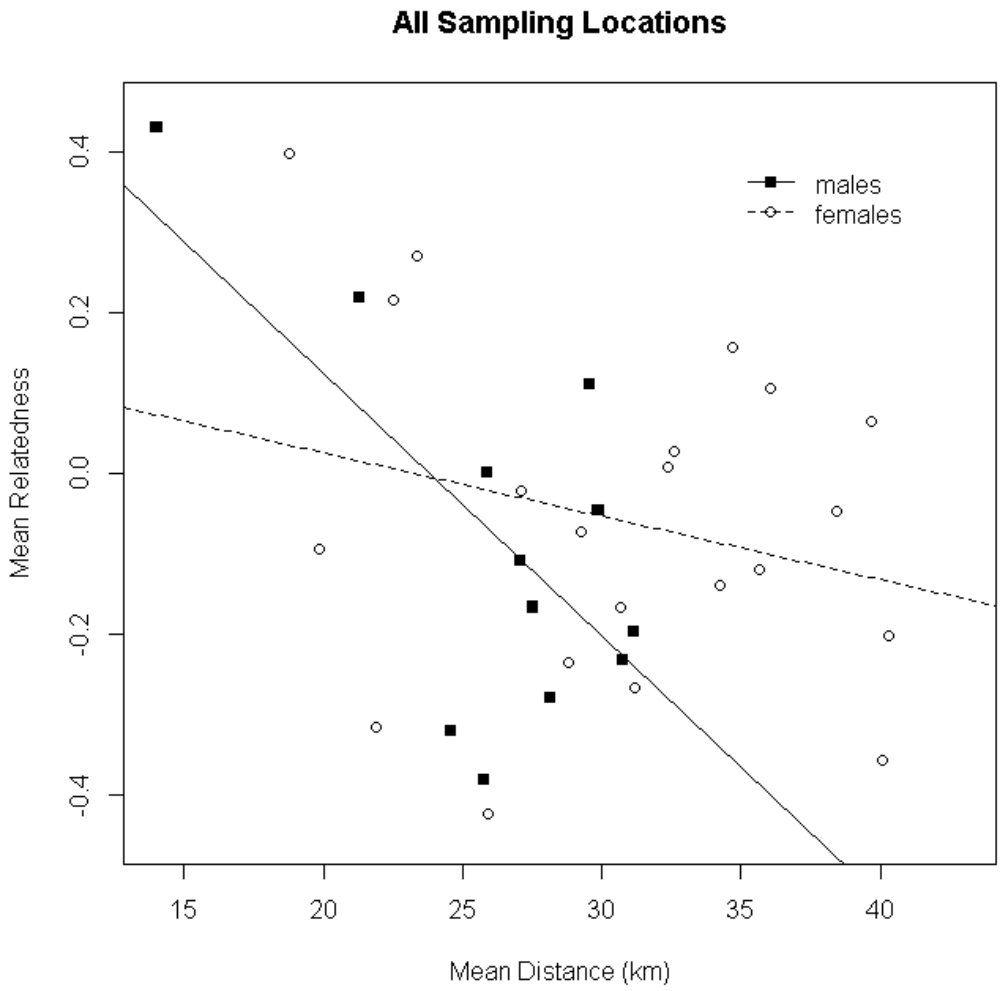

Figure 4.5: Regression on the mean ranked pairwise relatedness and mean geographic distance for samples collected from all locations in the study.

between males and females. The comparison in the variance of corrected assignment indexes was similarly inconclusive with no significant difference between males and females.

The calculation of both statistics (AI_C and F_{ST}) used in these tests are based on allele frequencies which require adequate sample sizes for accurate estimation. The small sample sizes in this study may be responsible for the ambiguity in these tests results. This is particularly evident in the example cited above in which only one female from the GGNP central region was sampled and the inclusion of this sample emphasized the lability of the F_{ST} values in the test.

The results of the Mantel and mean pairwise relatedness tests both showed geographical distance had a significant negative effect on the relatedness between males but not for females. This relationship was more apparent with the mean pairwise relatedness test than it was with the Mantel test. When Ngel Nyaki forest reserve was

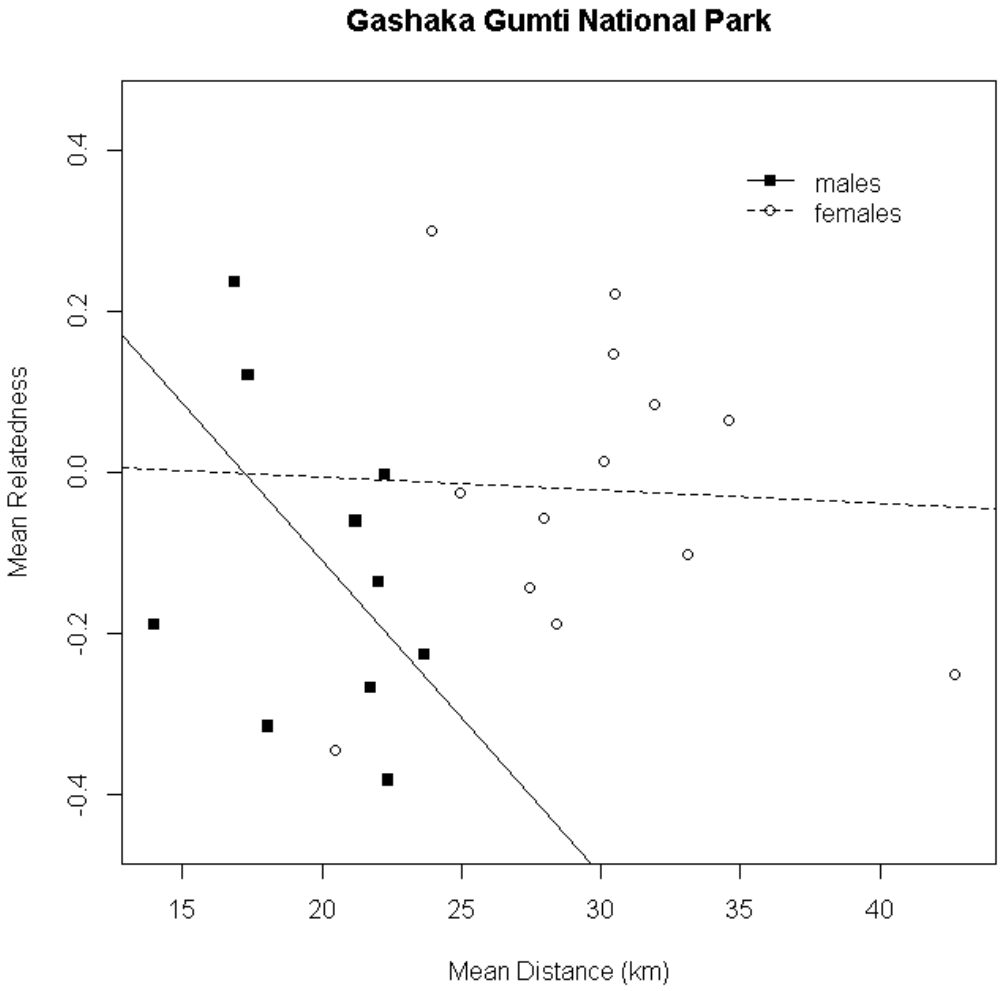

Figure 4.6: Regression on the mean ranked pairwise relatedness and mean geographic distance for samples collected in GGNP.

included in the analyses the relationship was weaker in contrast to analyses when it was excluded. This reduction in the strength of the relationship when including Ngel Nyaki forest reserve is due to the anomalous discovery of a male in GGNP central that potentially originated from Ngel Nyaki forest reserve (see Chapter three).

The mode of the distribution of the AI_C values was lower for females, when values were categorized, than it was for males which is the expected pattern under female biased dispersal. This pattern was not extremely pronounced however and the lowest AI_C value was that of the male sample collected in GGNP central. If this sample is a first generation migrant (as shown by the assignment test in Chapter three) then it poses a conundrum for the dispersal behaviour of the individuals in this area. The distance between the border of GGNP and Ngel Nyaki forest reserve (approximately 10-15 km) is well within potential territory sizes of chimpanzees (Wilson and Wrangham 2003). It may be that this individual was separated from the community at Ngel Nyaki during or after the construction of the road

that runs between Ngel Nyaki and GGNP and the increased human activity that inevitably entailed this development (Tukur *et al.* 2005). The road is believed to have been paved sometime since the 1970s and is still within the potential lifetime of a chimpanzee. It seems unlikely that the construction of the road would have created such an instantaneous barrier preventing him from returning but perhaps the forest cover between the locations at the time of construction had narrowed to such a degree to cause this. The presence of industrial workers in other sub-species ranges is regularly associated with the presence of professional hunters providing food for workers (Kormos 2003, Thibault and Blaney 2003). Added hunting pressure and the disturbance created by the construction of a road might have been enough for this individual to flee further than his usual home range.

Another, and biologically more interesting scenario, is that males do disperse in this sub-species as well as females. Both the first generation migrants detected in this study (a male and a female) where collected approximately 60 km from their most probable population of origin as identified by GeneClass2. The identification of this male as a migrant warrants further investigation to determine if this event was a product of habitat fragmentation or what conditions in this region or sub-species influence male dispersal. One possibility is simply that the mother of this individual dispersed or was separated from the original community after conception. If males don't disperse in this sub-species then the provenance of this sample being Ngel Nyaki lends to the evidence that Ngel Nyaki forest reserve has become isolated from GGNP. If this result is the product of habitat fragmentation then it poses its own unique questions as to why or how this individual survived when males are known to face significant aggression from neighbouring communities (Boesch *et al.* 2008, Goossens *et al.* 2005)

As only one male was identified in Ngel Nyaki forest reserve during the time of the field season of this study it is unlikely that this incident was the result of eschewed sex ratios unless they have changed dramatically in recent history. The sex ratio in GGNP was only marginally biased towards females which would suggest that signatures of sex-bias in GGNP are not completely explained by this fact. The pairwise relatedness and Mantel tests do not require the assumption of a pre-defined population structure or relies on allele frequencies. Taking into account the sample sizes in this study and the lack of knowledge around the pre-existing population structure in the national park the pairwise relatedness and Mantel test may be more insightful than the F_{ST} and assignment tests. Thus a summary of the results from this chapter indicate that female dispersal is evident but the detection of a male migrant however leaves room for speculation that males in this sub-species might disperse which is not known to occur in other sub-species.

Chapter 5 Population Size and Viability Analysis

Introduction

The estimation of population size is central to the dogma of conservation biology. Population size underpins three of the IUCN red list's five criterions for classifying endangered organisms (IUCN 2013). Criterion one classifies organisms based on the percentage reduction in population size. Criterion four relates to the number of individuals remaining while criterion three is dependent on the number individuals and the trend in population size. Forces that influence population size can be classified as either deterministic or stochastic. Deterministic forces refer to the birth and death rates and their causes in a closed population as well as immigration and emigration in open populations. Whilst deterministic forces, such as habitat destruction or hunting, are often the cause of species declines, stochastic forces become increasingly relevant in the management of conserved population because their effects are harder to predict and they exert a greater influence in smaller populations (Shaffer 1981, Gilpin and Soulé 1986).

Stochasticity comes in various forms. Environmental stochasticity and natural catastrophes affect mortality at the level of the population. Mechanisms of environmental stochasticity can include parasites, disease, predators, competition and resource availability (Shaffer 1981). Demographic stochasticity affects populations through chance events in reproduction and mortality at the level of the individual and its impact is relative to the size of the population (Lande 1993). Demographic stochasticity is therefore analogous to allee effects as population growth becomes negative or unstable below a specific population size (Lande 1998a, Stephens and Sunderland1999). Studies on the relative contribution of demographic stochasticity to extinction risk suggest that its effect may be profound and often underestimated (Legendre *et al.* 1999, Melbourne and Hastings 2008).

Genetic stochasticity acts on populations via the deleterious effects of inbreeding, the loss of genetic diversity and mutation accumulation (Frankham *et al.* 2002, Keller and Waller 2002). Loss of genetic diversity and the rate of inbreeding depend on the effective population size (N_e), defined as “the number of individuals that would result in the same loss of genetic diversity, inbreeding or genetic drift if they acted in the manner of an idealized

population" (Frankham *et al.* 2002), which is often much smaller than the actual population size (Frankham 2005). Genetic diversity decays exponentially over generations in populations with a small N_e and evidence for the elevated extinction risk of small populations due to genetic factors is now becoming more substantiated (Keller and Waller 2002, Spielman *et al.* 2004, Frankham 2005, O'Grady *et al.* 2006).

How small is too small

Shaffer (1981) proposed the concept of a minimum viable population (MVP) that is "…the smallest isolated population having a 99% chance of remaining extant for 1000 years despite the foreseeable effects of demographic, environmental and genetic stochasticity, and natural catastrophes." To estimate a universal minimum number of individuals to mitigate the effects of demographic and environmental stochasticity across species is complicated because their relative effects are influenced by a number of factors including growth rate, carrying capacity and age structure (Lande 1998b). Average persistence times have been shown to have a geometric relationship with population size under demographic stochasticity alone (Shaffer 1987). This would suggest that demographic stochasticity is only a threat to populations numbering in their 10's to 100's (Shaffer 1987, Nunney and Campbell 1993). On this premise it is expected that environmental stochasticity is preponderant in populations greater than 100 in number (Lande 1988). However more recent modelling has suggested that this assumption may be incorrect because a component of demographic stochasticity, demographic heterogeneity, which is the variation in birth and death rates in individuals related to specific characteristics of the individual, is often mistaken for environmental stochasticity and plays an important role in persistence times (Melbourne and Hastings 2008).

Franklin (1980 cited by Frankel and Soulé 1981) suggested that the number of individuals required to mitigate the impact of detrimental genetic effects in the short term was 50. This number stems from a rule used by animal breeders that selection for performance and fertility can offset inbreeding depression if the change in the inbreeding coefficient is less than 1% per generation. This translates into an N_e of 50, with a minimum number of fifteen individuals in the least numerous sex (Frankel and Soulé 1981). To safe guard a population in the long term from the loss of adaptive evolutionary potential Franklin (1980 cited by Jamieson and Allendorf 2012) recommended an N_e of 500. Contention around this issue has provoked estimates of the necessary N_e for long term adaptive potential to be as high as 5000 (Lande 1995).

Estimates of the ratio of N_e to actual population size (N) have indicated that it can be as low 0.1:1 which means that actual populations need to be at least an order of magnitude

higher than estimates of N_e (Frankham 1995, Franklin and Frankham 1998). Estimates of MVP's on 102 vertebrate species using population viability analysis highlight a mean and median MVP of 7316 and 5816, respectively (Reed *et al.*2003). The mean MVP from only primate data used in this study is 9362. Traill *et al.* (2007) arrived at a similar median MVP of 4169 when a meta-analysis was applied to studies from 212 species.

In this chapter I estimate the population size of the community of chimpanzees at Ngel Nyaki forest reserve and use population viability analysis to address three questions about their fate under different management options. First, what is the probability of the community persisting, assuming it is isolated, under a range of possible current population sizes and sex ratios without any intervention? Second, how would translocating individuals over the next 65 years at different intervals alter the probability of persistence of the population? Third, how much dispersal is required between Ngel Nyaki forest reserve and Gashaka Gumti National Park (GGNP) to maintain the probability of persistence above 95% over the next 300 years?

The first and second questions explore the consequences of immediate management actions (or lack thereof) in Ngel Nyaki forest reserve. Translocating individuals has some inherent risks involved such as disease transmission, potential outbreeding depression and the translocated individual being attacked and killed by residents (Hedrick and Fredrickson 2010). The former two risks can be mitigated but increases the cost of employing the strategy. The third question addresses the necessary conditions that a wildlife corridor between Ngel Nyaki forest reserve and GGNP would have to foster to mitigate the extinction risk to an acceptable level in the long term without intensive management.

Methods

Population size was estimated using the program *Capwire* (Miller *et al.* 2005). This program uses a maximum likelihood method that performs well when the number of individuals in the population is less than one hundred and the capture frequency of individuals is heterogeneous. Unique genotypes collected in Ngel Nyaki forest reserve from two sampling occasions in 2012 (see Chapter two for more details on sample collection) were treated as separate individuals. To determine the probability that non-unique genotypes belong to the same individual, the probability of identity was determined in GenAlex 6.5 (Peakall and Smouse 2006) using the PIsibs formula (Waits *et al.* 2001).

Population viability analysis was conducted using Vortex ver. 9.99 (Lacy *et al.* 1995). Models were constructed using parameters extrapolated from the literature on various sub-species of chimpanzees in lieu of data on the Nigeria-Cameroon chimpanzee. Parameters

that were consistent between all models are listed below with their sources in the order they are requested in Vortex under the sub-heading 'Constant Parameters'. Not all parameters that are allowed in Vortex have to be input and parameters not listed were not included in the models. Each scenario was run 1000 times with a maximum of 1000 years simulated to estimate the distribution of extinction times and the probability of persistence (calculated as the number of iterations that remained extant at any particular year in the simulation). Extinction was defined as the complete loss of all individuals of one sex.

Models without intervention

To predict the probability of persistence without any intervention the population was modeled over a range of possible current population sizes (N) and number of males in the starting population (M). The first population size modeled (N = 9) was modeled with only one male. This corresponded to the total number of unique genotypes amplified during this study and their respective genders as determined using the amelogenin assay (see Chapter two for details on sex identification of the samples). This represents a worst case scenario for the community at Ngel Nyaki forest reserve. In all other models a range of values for M were modeled for each population size. The population sizes chosen were based on an estimate of population size calculated from nest counts (Beck and Chapman 2009), the estimate of population size from this study and the upper limit of the confidence interval for estimate of this study. The age structure of the population was assumed to be even. When the number of males in the starting population was one the median age of males (22) for that male was selected.

Models with translocation

For the four scenarios without intervention that had the lowest median times to extinction, probability of persistence was modeled with translocation of individuals into the population. For each scenario, translocation of a single individual was simulated at intervals of either five, ten or twenty years starting five years from now and continuing until 65 years from now. Translocated individuals were either exclusively male or female for all translocation events in that scenario.

Models with dispersal

To estimate the necessary level of dispersal to maintain a probability of persistence of greater than 95% over 300 years I used a range of initial population sizes (10,20 and 30) that approximate the distribution of population sizes at year 50 from the models with translocation. Population sizes at year 50 were selected because this would be a realistic

timeframe in which to create a habitat corridor to facilitate dispersal. The value of M in Ngel Nyaki forest reserve for all models was assumed to be 20% of the total population size and the sex ratio at GGNP was assumed to be 50:50, these are similar to the sex ratios observed in this study (see Chapter four for more details on sex ratios in the regions in this study). The initial population size of GGNP was set to 1000, the lower estimate of its current population size estimate (Morgan *et al.* 2011). A uniform age distribution was assumed for both populations. When the number of males was equal to two, one adult and one adolescent was selected.

I assumed a 95% survival of dispersers. The probability of successful dispersal per year from GGNP to Ngel Nyaki was investigated over the range of values 0.1%, 0.05%, 0.02% and 0.01% which is equivalent to one successful migrant per 1, 2, 5 and 10 years, respectively, when the population size of GGNP is equal to 1000. The dispersal rate from Ngel Nyaki to GGNP was set to 1% which is equivalent to one migrant per 3.33, 5 and 10 years when the population size is equal to 30, 20 and 10 individuals, respectively. Thus the dispersal rate is density dependent and increased or decreased accordingly to the population size for both populations. Age of dispersers was set between 11and 40 (Goodall 1986) and could only be female (Mitani *et al*. 2002).

Constant parameters

Inbreeding

The number of lethal equivalents was set to 2.1, as estimated by Ralls *et al.* (1988) using captive populations of chimpanzees. To the best of my knowledge this is the only estimate of the number of lethal equivalents in chimpanzees. The percentage due to recessive alleles was set to 50%. This figure has been estimated from large populations of *Drosphilia* but is expected to be less in small populations of endangered animals because the purging of deleterious alleles will reduce it in the wild. Therefore this is a conservative estimate (Hedrick 2002).

Reproductive system

The reproductive system was selected to be polygynous (Tutin 1979, Goodall 1986). The age of first offspring in females was set to 13, the mean age of first reproduction reported by Wallis (1997). The age of first reproduction for males was set to 13 as predicted by Goodall (1986). The maximum age of reproduction was 43, the mean maximum age of reproduction estimated from six locations (Emery Thompson *et al.* 2007). Maximum number of broods per year was set to one and the maximum number of progeny per brood was two

(Goodall 1986, Wallis 1997, Ely *et al.* 2006). Although triplets have been observed in captive populations (Ely *et al.* 2006), the frequency of their occurrence is so low that it is not able to be input into vortex. Furthermore, to the best of my knowledge it has never been observed in the wild. The sex ratio at birth was assumed to be 50:50.

Reproductive rates

The percentage of adult females breeding was calculated from the formula:

$$(IBI)^{-1}$$

where *IBI* is the inter-birth interval (Miller and Lacy 1999). The mean inter-birth interval of 5.15 years determined by Wallis (1997) resulted in an estimate of 19% of adult females breeding in any particular year. No environmental variation in the number of breeding females was included as this figure could not be estimated. The probability of one offspring or two was set to 97% and 3%, respectively (Ely *et al.* 2006).

Mortality rates

Vortex allows for specific mortality rates for all ages under the age of first reproduction. Beyond this one mortality rate is input for all adults. Mortality rates for individuals below the age of first reproduction (<13 years) were extracted directly from the life table of the chimpanzees at Gombe, Tanzania compiled by Hill *et al.* (2001). The mortality rates for Gombe were selected because they represent the largest data set on the mortality rates of chimpanzees in one region that has been constructed and mortality in this data set is reported to be due to natural rather than anthropogenic causes. This purpose of these models was to determine the viability as influenced by natural causes. The adult mortality rate was calculated by summing the number of individuals in all age classes from 13 up to 43 (the age of last reproduction in my Vortex models) from the life table of Hill *et al.* (2001) and summing the number of deaths in these age classes then dividing the total number of deaths by the total number of individuals. The standard deviation of the mortality rate at each age was calculated using the formula for the standard deviation from the binomial distribution and rounded up to the nearest percentage (Miller and Lacy 1999):

$$\frac{pq}{n-1}$$

where *p* is the proportion of individuals that died, *q* is the proportion of individuals that survived and *n* is the number of observations that the data is calculated from.

Mate monopolization

The number of males in the breeding pool was assumed to be 100%. Although dominance rank is related to mating success, lower ranking males sire young with younger females (Wroblewski *et al.* 2009). As only one individual was identified as a male in the community at Ngel Nyaki forest reserve in contrast to eight females (see Chapter two and four for more information), it is likely that, at most, only a few males exist in the population and thus all males will have opportunities to mate.

Carrying capacity

The carrying capacity of Ngel Nyaki forest reserve and GGNP was set to 300 and 3000 individuals, respectively. These may be well over the true carrying capacities of Ngel Nyaki forest reserve and GGNP but the purpose of the model was to determine the fate of the population with and without specific management actions, neither of which are likely to increase the population in the near future close to the carrying capacity. There are no estimates of the carrying capacity at Ngel Nyaki or Gashaka Gumti National Park therefore arbitrarily large values were chosen.

Results

Nine unique genotypes were identified from the samples collected from Ngel Nyaki forest reserve over the duration of the whole study. Seven of those nine unique genotypes were collected once each, and the other two were both collected five times each. However not all of the five samples for each of these two genotypes were successfully amplified at all eight loci. The range of matching genotypes across all loci for these samples was between four and eight which corresponded to PIsibs values of 0.019 and 0.001, respectively. The estimated population size was 21 (lower CI = 9, upper CI = 31). Under demographic factors alone the population growth rate in all models was stable. The exponential growth rate (r) was 0 and the annual growth rate (λ) was 1 therefore the number of births in the population matched the number of deaths and under these two forces alone the population size would be constant.

Models without Intervention

The range of median times to extinction were between 53 (N = 9) and 264 years (N = 31, M = 5, Figure 5.1), whilst the range of extinction times were between one year (N = 9) and year 971 (N = 31, M = 5). When N = 9 and N = 12, the median times to extinction were always below 100 years irrespective of the value of M. The scenario with the greatest median time to extinction of 264 years occurred when N = 31 and M = 5. When N = 21 the longest median time to extinction was also when M = 5 (Figure 5.1).

All models estimated the population to be extinct by year 1000 (Figure 5.2). By year 50 the probability of persistence ranged between 0.591 (N = 9) and 0.999 (N = 31, M = 5). For both N = 9 and N = 12 the probability of persistence was below 0.95 by year 17 and below 0.05 by year 246. By year 100 the probability of persistence for all models except when N = 31 M = 5 was below 0.95 and by year 600 all models had a probability of persistence of less than 0.05.

Models with translocation

Probability of persistence and median times to extinction of the population at Ngel Nyaki forest reserve always improved more with the translocation of females than males, thus the results of the models with male translocation are not discussed further. The four

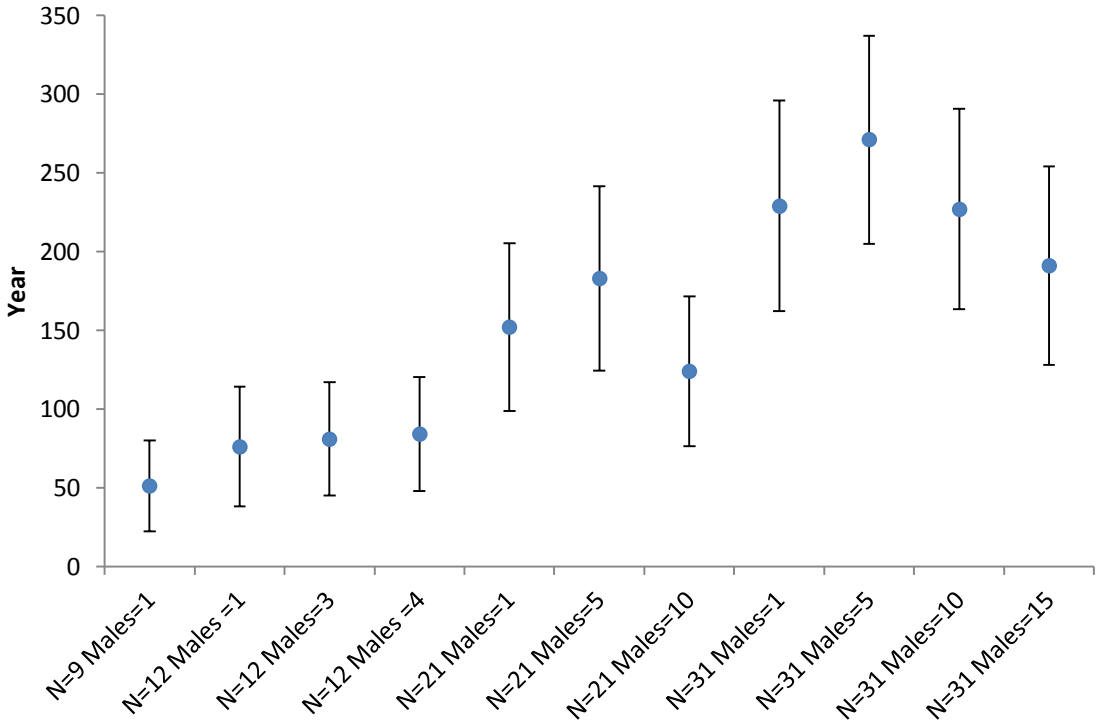

Figure 5.1: Median times to extinction in models with different initial population sizes and sex ratios without any simulated intervention measures. The population sizes (N) and number of initial males (Males) modelled are on the x axis.

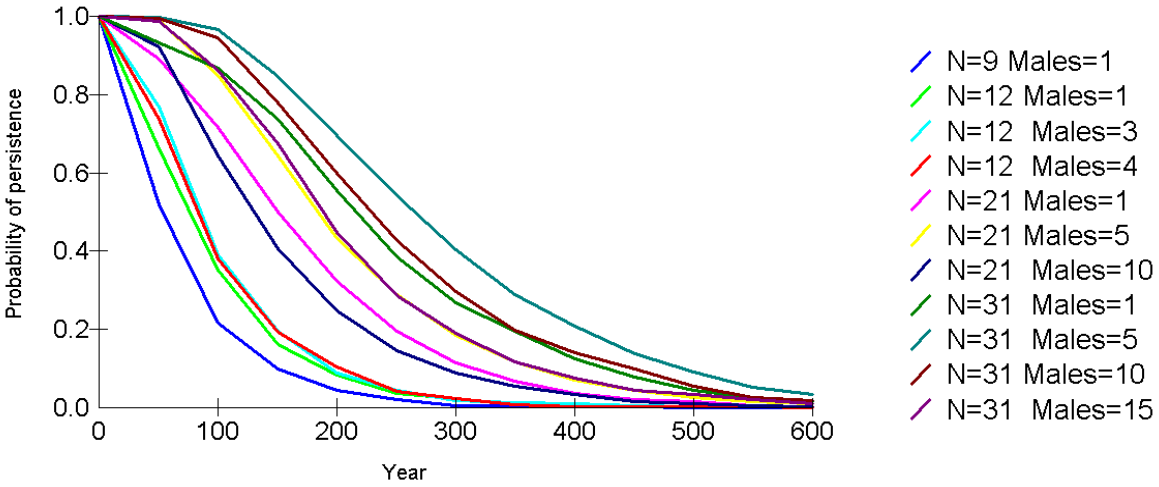

Figure 5.2: Probability of persistence for all models without any form of intervention for different population sizes (N) and number of initial males in the starting population (Males).

models without intervention that had the lowest median times to extinction were N = 9, M = 1 and N = 12, M = 1, 3 and 4. The average increase in the median time to extinction for these four models when translocation was simulated at intervals of five, ten and twenty years were 168, 91 and 52 years, respectively. Probability of persistence increased with more frequent translocation in all models (Figure 5.3). By year 65 (the last year of translocation) the probability of persistence was greater than 0.9 when N = 12 and M = 3 or M = 4. In the other two models (N = 9 and N = 12, M = 1) the probability of persistence was below 0.8 by year 65.

Models with dispersal

Two of the twelve models including dispersal had a probability of persistence greater than 0.95 for 300 years (Figure 5.4). These were when N = 20 and N = 30 and percentage of successful annual dispersal was 0.1%. For all models where N = 10 the probability of persistence had dropped below 0.95 by year 17. When the percentage of successful annual dispersal was 0.05 and the probability of persistence dropped below 0.95 by year 160 and year 84 when N = 30 and N = 20, respectively (Figure 5.4). When the percentage of successful annual dispersal was 0.02 or 0.01 the probability of persistence dropped below 0.95 and declined relatively rapidly following year 65 when N = 20 and year 96 when N = 30 (Figure 5.4).

Discussion

The population viability analysis shows that without intervention the possibility of local extinction of the community of chimpanzees at Ngel Nyaki is an impending concern. The worst case scenario, that one male and eight females exist in the reserve, places the community in immediate danger of extinction. If this is the true scenario, then the survival of

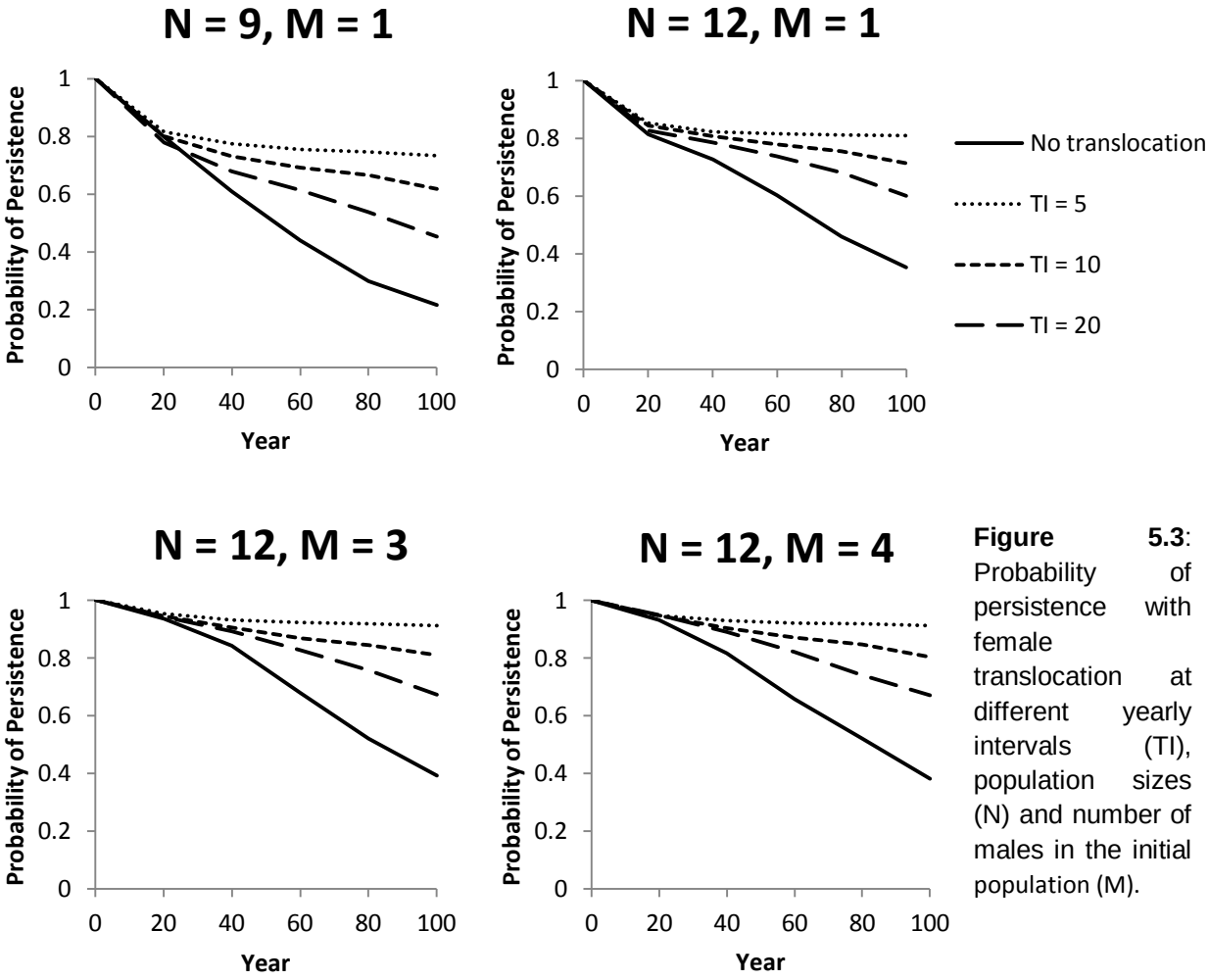

Figure 5.3: Probability of persistence with female translocation at different yearly intervals (TI), population sizes (N) and number of males in the initial population (M).

the population is dependent on that one male. However reports of sightings and vocalizations from other researchers in the reserve suggest that population sizes of 12 or 21 are both plausible scenarios. If the latter population size is true and more than one male exists, then the community may be stable for the near future. In Bossou, Guinea the population of chimpanzees has remained stable at 20 individuals for over 26 years (Sugiyama 2004).

The models without intervention highlight the importance of the sex ratio in the persistence of the population at Ngel Nyaki. In the models with varying numbers of males in the initial population, the ideal number of initial males is five when the population size is twenty one or thirty one. When the number of males exceeds this, it reduces the probability of persistence and median time to extinction. The probable explanation for this is the synergy of female controlled growth rate and long inter-birth intervals. The long inter-birth interval means that the number of females in the breeding population is roughly 20% at any one time. Therefore there has to exist greater than 25 females for there to be more than one female in estrous to every male if five males exist. The range of initial population sizes only

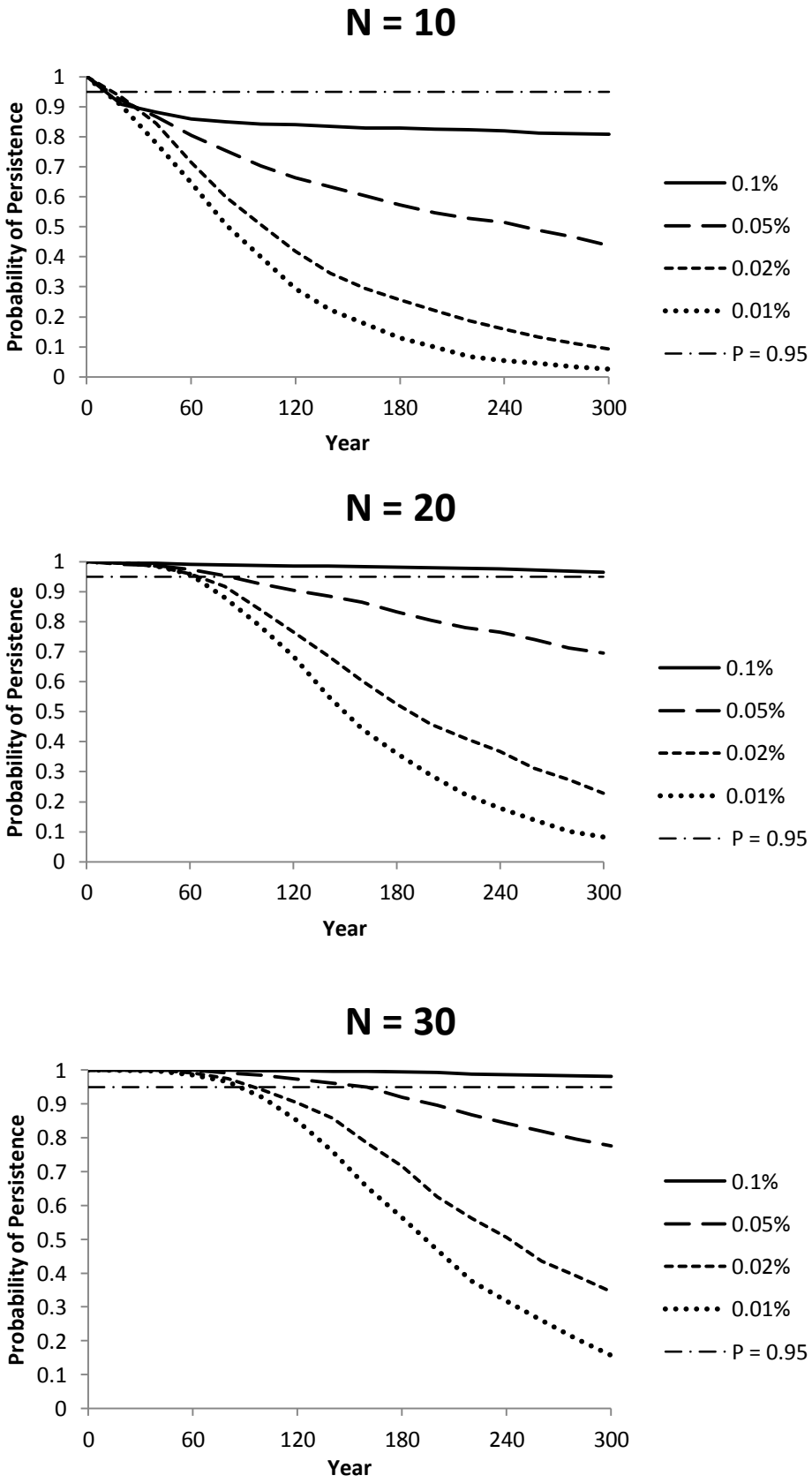

Figure 5.4: Probability of persistence of the population at Ngel Nyaki forest reserve when different dispersal rates from GGNP to Ngel Nyaki forest reserve are modelled for different initial population sizes (N) at Ngel Nyaki forest reserve.

just exceeds this in the scenarios explored. Even when it does exceed this value, the polygynous mating system modeled in these scenarios permits males to sire offspring with more than one female. The negative effect of inbreeding from fewer males is outweighed by the relative increase in population growth by the biased sex ratio.

Translocation of females is a potential solution to maintaining the population in the short term. The most frequent interval between translocations modeled in this study (every five years) resulted in a probability of persistence of approximately 0.9 for the first 100 years if the initial population size is 12 and there are three or four males. If there is only one male then the probability of persistence with this level of translocation drops to approximately 0.75-0.8 when the population size is either nine or twelve. Iterations with rapid extinction times in these latter two models are presumably due to the death of the solitary male before male offspring are produced resulting in the obsolescence of female supplementation. Despite female supplementation faring better than male supplementation in all models the catastrophic loss of that male before siring male offspring is obscured by the use of median times to extinction and averaged probabilities. If one male exists then male supplementation will be a more salient proposition.

The results of the dispersal models show that if a probability of persistence of 0.95 is desirable, the necessary dispersal rate predicted by these models is equivalent to 1 migrant per year if the population size is greater than twenty, a perhaps unrealistic target in the near future. The efficacy of these models, however, is dependent on several key parameters two of which had to be assumed; namely, the survival rate of dispersers and the dispersal rate out of Ngel Nyaki. The survival rate of the dispersers is crucial to the validity of the models. If this survival rate cannot be achieved then the overall effect is comparable to a reduced dispersal rate and therefore a reduction in the average probability of persistence. Any attempt to create a corridor would seem rather futile without attempting to monitor factors, such as hunting pressure, that might affect the survival rate. The dispersal rate out of Ngel Nyaki is unlikely to be as critical. In these scenarios it is modeled as percentage of the population size hence it increases with increasing population size here.

Possibly the most significant result to come from these models is the time frame which the demography of the population accords for action. If only one male exists then local extinction is an imminent possibility but if several males exist and the population is greater than twenty then no immediate action may be required. The models also identify sex ratios that would maximize the probability of persistence. Conservation targets for the community at Ngel Nyaki should endeavor to maintain a population of at least five males and approximately 16 or more females. The most pressing subsequent activity should be to

estimate a more precise population size and sex ratio. Conservation strategies are discussed in more detail in Chapter six.

Chapter 6 Conclusions and Conservation Strategy

Conclusions

The level of genetic differentiation between the community of chimpanzees in Ngel Nyaki forest reserve and the three regions analysed in Gashaka Gumti National Park (GGNP) was similar to the level of genetic differentiation among locations within the national park alone (see Chapter three for more detail). All pairwise F_{ST} values were significant which suggests these regions and Ngel Nyaki may potentially contain distinct communities of chimpanzees. While the F_{ST} values on their own are not sufficient to imply Ngel Nyaki forest reserve is isolated from GGNP when the assignment tests are considered as well there is more evidence for this. The assignment tests show a modest tendency for samples collected in Ngel Nyaki forest reserve to generally have higher log likelihood values for Ngel Nyaki than that of GGNP and vice versa. This pattern is not apparent between pairwise comparisons of log likelihood values for the regions within GGNP. For this pattern to occur Ngel Nyaki forest reserve may have only recently become isolated from GGNP. A combination of the paving of the road running between Ngel Nyaki forest reserve and GGNP sometime since the 1970s and a steep increase in human population pressure since then (Tukur *et al.* 2005) are a credible cause for this isolation to have occurred. The road is the only paved road on the whole of the Mambilla Plateau and connects to the border with Cameroon. Regular traffic passes along the road in the daytime and human settlements are more common in the vicinity of the road than further away. Arable and cattle farming are both pervasive in the region between Ngel Nyaki forest reserve and GGNP. These facts and the timorous behaviour of chimpanzees in the region in response to human activity witnessed during this study and reported by local residents discourage the notion that a chimpanzee might endeavour to navigate this landscape.

The discovery of a male migrant in the centre of GGNP that probably originated from Ngel Nyaki poses an enigmatic scenario. If this individual were to have purposefully migrated from Ngel Nyaki to GGNP, it would present a fascinating opportunity to examine the ecological conditions that alter the pattern of female biased dispersal that is typically observed in chimpanzees (Mitani *et al.* 2002). Nevertheless a number of other scenarios could have led to the presence of an individual with this genotype in GGNP. The mother

may have migrated post conception from Ngel Nyaki to GGNP or was potentially a resident of GGNP that transferred to Ngel Nyaki temporarily, conceived this individual and returned to GGNP prior to extensive human presence in the region. Temporary intergroup transitions of this nature are not unknown in chimpanzees (Goodall 1986). Alternatively this finding may simply reflect sampling error; the individual in question may have originated from an unsampled community with allele frequencies similar to that of the community at Ngel Nyaki or the allele frequencies calculated in this study are inaccurate. The small number of samples successfully amplified for sufficient DNA to be included in this study does limit the strength of the conclusions that can be drawn. Notwithstanding this sample, the tests for sex biased dispersal conducted in this study suggest female chimpanzees are the predominant dispersers in the region.

The sex ratio of the community at Ngel Nyaki of one male to eight females observed during this study elicits concerns about the immediate future of the community of chimpanzees at Ngel Nyaki forest reserve. Reassuringly, small populations of chimpanzees have been known to persist for periods of over twenty years (Sugiyama 2004) and in one instance an introduced island population increased from 17 to 40 individuals (Goossens *et al.* 2003). If only one male does truly exist then the population at Ngel Nyaki faces an immediate risk of local extinction due to demographic stochasticity. Reports that hunting has occurred recently within Ngel Nyaki forest reserve exacerbates this concern (Ogunjemite and Ashimi 2010). The forest is protected and game guards conduct regular patrols in the region but it is impossible to guarantee the security of these extremely elusive animals. Inbreeding in the population may not have been apparent during this study but inbreeding depression may develop in future generations.

Translocation as a conservation strategy

The effect of immigration into populations has been shown to increase persistence times considerably (Stacey and Taper 1992). In this study models of female supplementation, on average, increased median times to extinction by 52 – 168 years depending on the frequency of reintroduction (see Chapter five). Since the possibility of Ngel Nyaki forest reserve being isolated from GGNP cannot be discounted, and there does not exist any known populations of chimpanzees within 30 km in any other direction, translocation of chimpanzees into Ngel Nyaki forest reserve may be a suitable strategy to ensure the viability of the community. Translocation can alleviate inbreeding, maintain or boost genetic diversity, bolster population sizes and increase the growth rate of the population (Goossens *et al.* 2002). This strategy involves certain risks and implications which need to be weighed against the potential benefits before being implemented.

Genetic and ecological similarity

Translocated individuals should be genetically similar to the recipient populations (IUCN/SSC 2013). This helps to avoid outbreeding depression and maintain evolutionary significant units (Moritz 1999, Goossens *et al.* 2002, IUCN/SSC 2013) but may sacrifice the opportunity to increase evolutionary adaptive potential. Individuals that are genetically similar are also more likely to have comparatively similar behavioural and ecological traits. Similarity in these traits could increase the probability of survival of translocated individuals in the range of the recipient populations. Individuals with similar ecological requirements probably have comparable dietary habits, the sources of which are more likely to be available in the habitat of the recipient population.

To satisfy these criteria the most logical provenance of an individual for translocation to Ngel Nyaki forest reserve would be GGNP. The identification of a potential migrant in this study is evidence that historical migrations have occurred between the two locations and translocating an individual who originated from GGNP would be akin to restoring natural gene-flow. Confiscated chimpanzees in the Limbe Wildlife Centre, Cameroon, have already had their origins traced back to GGNP (Ghobrial *et al.*2010) which indicates that potential candidates for translocation are already in captive populations.

Which individual to translocate

The life history of chimpanzees raises another consideration. Chimpanzees have a long infancy (approximately 4 years) and during this time they learn the necessary behaviours to fend for themselves in the wild. It is not until they are approximately six to ten years old that they become independent. Candidates for translocation should therefore be wild born individuals that have the behavioural capacity to survive in the wild (Goossens *et al.* 2002, Britt *et al.* 2004).

The sex of the translocated individual is ideally female in chimpanzees (Goossens *et al.* 2005b). A translocation project that released 37 chimpanzees into the wild recorded three deaths from 27 released females (Goossens *et al.* 2005b). Only two of ten released males are known to have died but this number would have been much greater without intervention as many males were attacked but received veterinary care. This result is consistent with behavioural observations on wild communities that females can disperse more easily into neighbouring territories whiles males are more likely to be met with aggression from neighbouring males (Wilson and Wrangham 2003, Boesch *et al.* 2008).

Goossens *et al.* (2005) recommend female adolescents for translocation to increase the probability of survival.

This provides an additional complication to managing the viability of the population of at Ngel Nyaki forest reserve as demographic stochasticity, particularly the lack of males, is potentially the most serious threat. If several males exist but remain unsampled at present, then female translocation is a clearly more suitable strategy (see Chapter five for more detail). However if there is a lone male and he does not sire offspring, or his offspring do not survive to reproduce then male supplementation is essential to the continued existence of the community. Therefore further investigation into the exact sex ratio is an immediate precursor to deciding which strategy to implement. Given the high mortality of male translocations reported by Goossens *et al.* (2005), the most cautious strategy, if male supplementation were to be required, would be to monitor the population closely at Ngel Nyaki forest reserve and only attempt male supplementation once it is confirmed that the existing male has died and no more are present.

Disease

The greatest risk of employing translocation is the threat that the translocated individual poses to the recipient population through the introduction of pathogens (Goossens *et al.* 2002). Natural diseases such as malaria and anthrax are known to occur in certain parts of the range of the Nigeria-Cameroon chimpanzee (Morgan *et al.* 2011). Chimpanzees are believed to be susceptible to pathogens originating in humans (Pusey *et al.* 2008). During the process of translocation the chimpanzee will necessarily come into close proximity, if not contact with humans. To minimize the risk of disease transmission an individual needs to be vaccinated for pathogens known to inflict chimpanzees. Quarantining individuals at Ngel Nyaki before introduction would be advisable but without any infrastructure in place to hold chimpanzees it may be unfeasible. Furthermore chimpanzees are reported to become highly stressed following transportation if they are not released immediately. This is obviously detrimental to their welfare and may reduce their likelihood of survival if they panic and flee when released (Goossens *et al.* 2002).

Suitability of Ngel Nyaki

One more consideration is the suitability of Ngel Nyaki for translocation. The carrying capacity of the forest is unknown and so there exists the possibility that the population is already limited by the level of food resources in the forest. Estimates of the total number of chimpanzees that Ngel Nyaki forest reserve can realistically support would provide valuable data in guiding management actions. As the research station for the Montane Forest

Conservation Initiative is located next to the forest and numerous ecological studies are in progress or have been completed there, ecological data that will assist in calculating this may already be available. The location of this research station also provides the capacity for long term monitoring to be carried out by trained field assistants to assess the success of translocation.

Management and conservation

The conservation plan for the community of chimpanzees at Ngel Nyaki forest reserve outlined here is designed to manage the population in respect to the negative effects of genetic and demographic stochasticity and to maintain the genetic diversity. The evidence supporting the actions necessary to conserve the population is summarized below.

Mitigating the effect of demographic and genetic stochasticity

Models of population persistence without human intervention showed significant increases in median times to extinction when the size of the community greater than twenty one (see Chapter five for more details). In these models, the highest median times to extinction occurred when there were five males in contrast to one, ten or fifteen males. To palliate the effect of demographic and genetic stochasticity and maximise population persistence times, five males and 26 females would be the optimal size and sex ratio as predicted by the parameters modelled in this study. If the reproductive and mortality rates extrapolated from the literature on the other sub-species of chimpanzee accurately reflect the demographic parameters of the community of chimpanzees at Ngel Nyaki forest reserve then population growth is stable and the population will not increase without intervention.

Maintaining 90% of genetic of diversity

To ensure the viability of the population, genetic diversity should be monitored and maintained. The expected heterozygosity of the community of chimpanzees at Ngel Nyaki forest reserve was 0.711. A target of conserving 90% of natural genetic diversity for 100 years has been recommended (Frankham *et al.* 2002). This is based on the prediction that human population pressure on the natural environment will decline by that time as the human population growth rate slows or declines. Conserving 90% of this genetic diversity would result in an expected heterozygosity in 100 years of 0.6399. If this could be achieved, 100 years would also be a plausible timeframe to create a habitat corridor between Ngel Nyaki forest reserve and GGNP to re-establish natural gene-flow.

I estimated the required effective population size to maintain this level of genetic diversity using the formula:

$$\frac{Ht}{Ho} = (1 - \frac{1}{2Ne})^{t} \sim e^{-t/2Ne}$$

where *Ht/Ho* is the change in heterozygosity, *t* is the number of years divided by generation time and *Ne* is the effective population size (Frankham *et al.* 2002). A chimpanzee's generation time is approximately 25 years (Langergraber *et al.* 2012) so the effective population size required to maintain 90% of the heterozygosity for 100 years is 18.9 individuals.

The question remains what is the ratio of the effective population size to the actual population size? The effective population size is influenced by fluctuations in population size, the family size and the sex ratio (Frankham *et al.* 2002). As there is no data on the family size or historical population sizes for the community of chimpanzees at Ngel Nyaki I modelled the effective population size based on a range of possible population sizes and sex ratios using the formula:

$$N_e = \frac{4N_{ef}N_{em}}{N_{ef} + N_{em}}$$

where N_e is the effective population size, N_{ef} is the effective number of breeding females and N_{em} is the effective number of breeding males (Frankham *et al.* 2002). I estimated the effective number of breeding males and females in the population by assuming an even age structure and calculating the proportion of individuals that are past adolescence. As chimpanzees have little or no post reproductive lifespan I subtracted the age at first reproduction, assumed here to be 13 (Goodall 1986), from the life expectancy to obtain the number of mature years in a chimpanzees lifespan, and divided this by the life expectancy to derive the expected proportion of adults. I multiplied this proportion by the number of males or females being modelled to calculate the effective number of mature breeding adults for each sex. Reports on other sub-species indicate that chimpanzees live and reproduce into their forties but this can vary (Emery Thompson *et al.* 2007) thus I estimated the effective population size for three possible life expectancies, 30, 35 and 40 years (see Figure 6.1).

The population sizes chosen are based on the number of unique genotypes identified in this study (nine, consisting of one male and eight females), the population size based on nest counts (12, Beck and Chapman 2008), the population size estimated in this study (21) and the upper limit of the confidence interval of this estimate of this study (31).

According to these models the only scenarios where the effective population size exceeds that of the required amount to maintain the genetic diversity is when the population

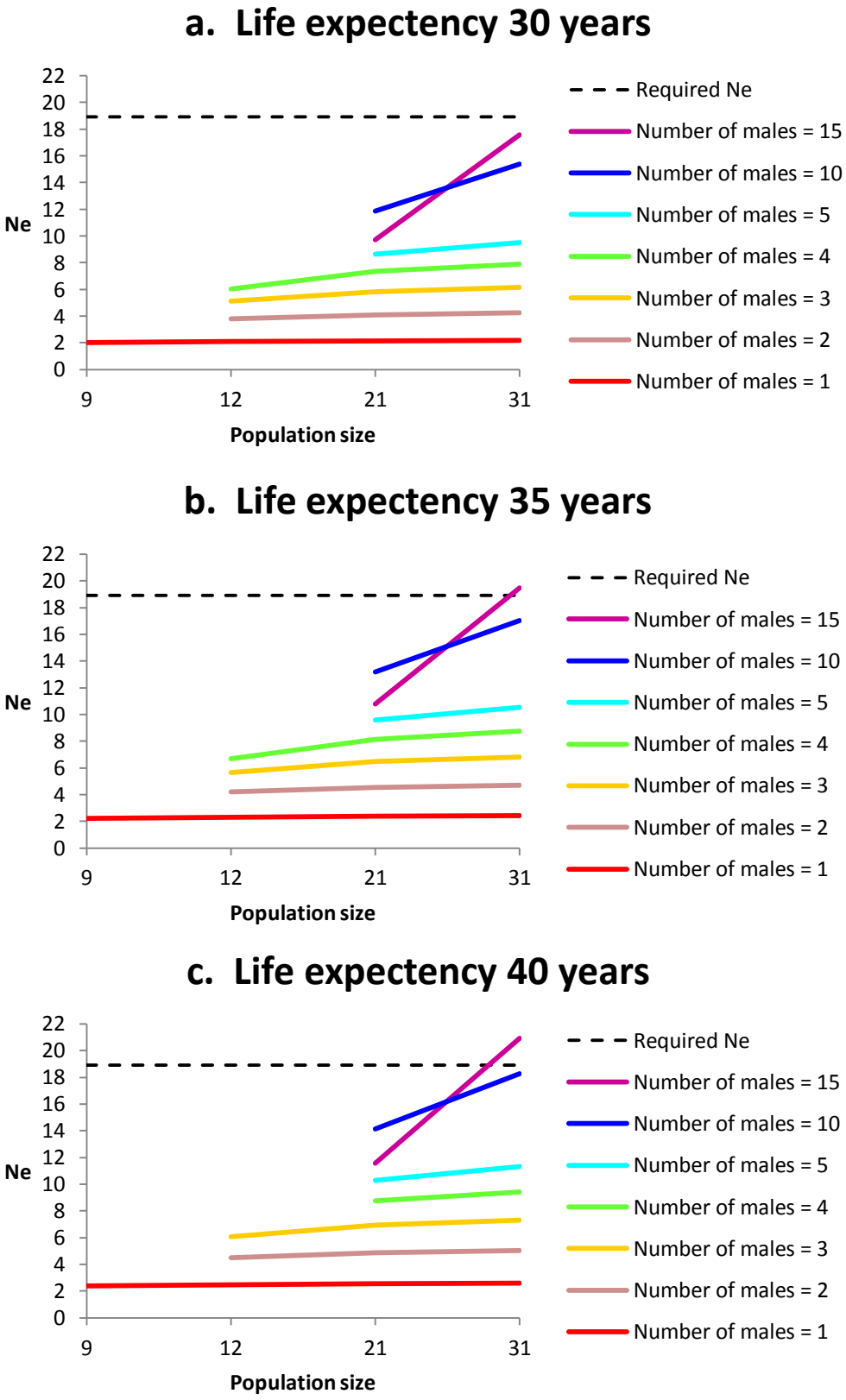

Figure 6.1: The effective population size of the community of chimpanzees at Ngel Nyaki forest reserve as modelled for a range of different potential population sizes and sex ratios when the life expectancy of a chimpanzee is assumed to be a) 30 years b) 35 years or c) 40 years. The required effective population size to retain 90% of the genetic diversity estimated during this study for 100 years is indicated by the black dashed line.

size is thirty one, the number of males and females is approximately even and the life expectancy is 35 or more (see Figure 6.1).

Conservation plan

The optimal population size to maximise population persistence times and maintain 90% of the genetic diversity is thirty one individuals or more. Population persistence times are extended when the number males is five in contrast to the effective population size being highest when there are 15 males and 16 females. If the highly biased sex ratio observed in this study (one male and eight females) accurately reflects the true sex ratio, increasing the population size to this level and incorporating fifteen males is unrealistic due to the high mortality associated with male translocations and the number of translocations that would be required. Genetic diversity can be increased by augmenting the population by translocating genetically dissimilar individuals. The number of potential current scenarios were the effective population is so low that the genetic diversity will theoretically deteriorate exceedingly rapidly is high, suggesting that intervention is necessary if 90% of the genetic diversity is to be maintained. Augmenting the population with the translocation of females will have the multiplicative advantage of increasing the growth rate, population size and genetic diversity of the population. As the cost of translocation is very high and translocating individuals could have potentially catastrophic consequences through the introduction of disease the heuristic strategy would be to translocate as few individuals as possible to sustain a positive population growth rate and maintain the genetic diversity.

The specific goals for the conservation of the chimpanzees at Ngel Nyaki forest reserve are:

1. Maintain a positive growth rate with the aim to increasing the population to thirty one individuals with at least five males.
2. Maintain 90% of the genetic diversity.

The specific actions and objectives for achieving these goals are listed below and a flow chart for the conservation plan is presented in Figure 6.2.

Estimate the carrying capacity of Ngel Nyaki forest reserve.

The carrying capacity will determine the maximum population size that is possible at Ngel Nyaki forest reserve. If the estimate is lower than the target population size above then it should be adjusted to match the carrying capacity. If sufficient data exists to calculate this then the carrying capacity can be estimated at no further cost. If however more data is required trained field assistants at the Montane Forest Conservation Initiative can be used to collect the data.

Conduct a genetic survey, beginning as soon as possible and continuing once every 5 years.

An intensive genetic survey will allow a more precise sex ratio of the population to be determined and provide a minimum population size. If no male individuals are detected then the population can be augmented by male translocations. Expected heterozygosity can be calculated to monitor the genetic diversity in the population. Due to the high cost associated with genetic surveys, an interval of five years is recommended to detect changes in sex ratio as females give birth approximately every five years (Wallis 1997). Therefore at the time of each genetic survey all or most of the females would have had an opportunity to reproduce. As the size of the community at Ngel Nyaki is expected to be small (approximately 10 – 20 individuals based on the estimates of this and previous studies) then only a few loci need to be amplified to obtain an adequately small probability of identity of individuals to assess the population size using genetic mark recapture methods and to keep costs low. The five loci used in this study with the greatest number of alleles (range seven to nine) yield a PI sibs value of <0.01 when used to identify individuals. The cost of a genetic survey employing these five loci is approximately $8500 USD.

Conduct biannual nest count surveys.

Biannual nest counts will allow fluctuations in the population size to be monitored at relatively lower cost than conducting genetic surveys. If there is a significant drop in the population size as estimated by the nest counts, female supplementation can be initiated to boost the population. If the population is stable or growing then no action may be required and continual monitoring can be conducted. Full time field assistants at the Montane Forest Conservation Initiative can perform biannual nest counts.

Translocation

If the above actions are followed and the results call for the translocation of an individual then suitable candidates can be screened. There are four sanctuaries in the range of the Nigeria-Cameroon chimpanzee but only one of these, the Drill Ranch, is in Nigeria (Morgan *et al.* 2011). This would be the best location from which to source an individual for translocation. A set of 10 microsatellite loci has been used in previous studies to identify the source of origin of captive chimpanzees in the range of the Nigeria-Cameroon chimpanzee (Ghobrial *et al.* 2010). Individuals are quarantined on arrival at the Drill Ranch but would need to be vaccinated before being suitable for reintroduction. Translocations would need intensive post-release monitoring to judge whether the strategy had been a success. The criteria for success, for any scenario requiring translocation, would be the survival and reproduction of the translocated individual. If the translocation was conducted in response to

declining growth rate or expected heterozygosity then the criteria for success would also include the reduction in decline of either factor.

Radio collars can be used to monitor the survival of the individual. A genetic survey should be conducted twelve months post release to see if the individual has reproduced and what the sex of the offspring is. Goossens *et al.* (2005) report that translocations cost approximately $50,000 USD per individual that survives.

Additional actions and conservation measures

Additional to these actions camera traps should be placed near fruiting trees such as members of the genus *ficus* which the chimpanzees are known to feed on. Hides could also be constructed to facilitate observations. These measures will help to ascertain the age structure of the population which has been assumed here and used to calculate the number of individuals in the effective breeding population. Extensive conservation measures as proposed here would be futile if poachers enter the reserve and remove more individuals. It is unlikely the population can survive any level of harvesting. A concerted effort to raise awareness in the region to the dangers of eating chimpanzee meat, i.e. disease transmission, and the importance of the animals to the natural heritage of Nigeria should be conducted in unison with the conservation measures outlined above. Surveys of residents in the towns where markets have been known to be selling chimpanzee meat (Ogunjemite and Ashimi 2010) can be used to gauge the awareness of local residents to the dangers of eating chimpanzee meat. Poster campaigns highlighting these dangers and post campaign surveys could monitor the effectiveness of such methods.

Education is often cited as the key to effecting long lasting change (Morgan *et al.* 2011). The Montane Forest Conservation Initiative already hosts a number of schools for environmental awareness programs. Actively encouraging participation of schools in the vicinity of GGNP and Ngel Nyaki forest reserve and extending these educational programs to other groups that include adult members who are more likely to participate in the purchasing of chimpanzee meat may also help to discourage this practice. Subsidy based conservation measures could also be initiated in locations adjacent to chimpanzee habitat. Farmers who preserve rather than destroy the forest can be financially supported for their efforts. This method would help to instil a perception of value in keeping chimpanzees alive. In regions such as Akwaizantar, which is located 30 km south of Ngel Nyaki, rural inhabitants practice slash and burn agriculture. The region has a title of forest reserve but is extremely remote and the authorities have no presence there. Without attempting to change perceptions and attitudes of the local residents to the animals in such impoverished remote areas the decline in numbers is unlikely to change due to the financial advantage associated

with the destruction of chimpanzee habitat or slaughter of the animals, and the absence of authorities to deter these practices.

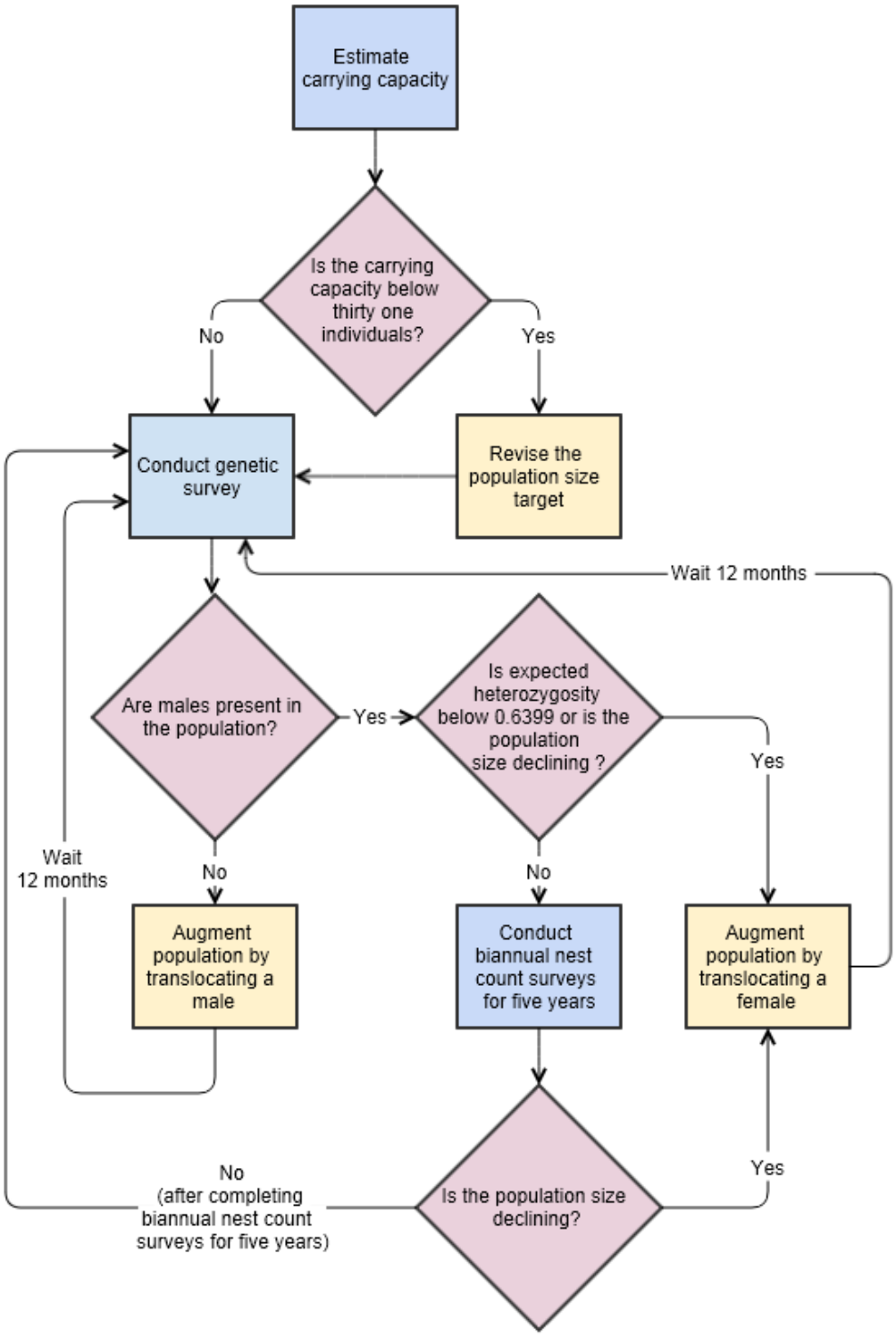

Figure 6.2: Flow chart for the conservation plan to mitigate the effects of genetic and demographic stochasticity and conserve 90% of the genetic diversity observed during this study.

References

Abwe EE, Morgan BJ. 2008. The Ebo forest: Four years of preliminary research and conservation of the Nigeria-Cameroon chimpanzee (*Pan troglodytes vellerosus*). *PanAfrica News* 15(2):26.

Anderson DP, Nordheim EV, Boesch C, Moermond T. 2002. Factors influencing fission-fusion grouping in chimpanzees in the Taï National Park, Côte d'Ivoire. In: Boesch C, Lohmann G, Marchant L, editors. *Behavioural diversity in chimpanzees and bonobos*. Cambridge: Cambridge University Press. pp 90-101.

Arcadi A, Robert D, Boesch C. 1998. Buttress drumming by wild chimpanzees: Temporal patterning, phrase integration into loud calls, and preliminary evidence for individual distinctiveness. *Primates* 39(4):505-518.

Arnhem E, Dupain J, Vercauteren Drubbel R, Devos C, Vercauteren M. 2007. Selective Logging, Habitat Quality and Home Range Use by Sympatric Gorillas and Chimpanzees: A Case Study from an Active Logging Concession in Southeast Cameroon. *Folia Primatologica* 79(1):1-14.

Balcomb SR, Chapman CA, Wrangham RW. 2000. Relationship between chimpanzee (*Pan troglodytes*) density and large, fleshy-fruit tree density: Conservation implications. *American Journal of Primatology* 51(3):197-203.

Ballou J, Ralls K. 1982. Inbreeding and juvenile mortality in small populations of ungulates: a detailed analysis. *Biological Conservation* 24(4):239-272.

Barnwell R. 2011. Exploring and Protecting West Africa's Primates: The Gashaka Gumti Primate Project in Context. In: Sommer V, Ross C, editors. *Primates of Gashaka*: Springer. pp 39-54.

Beck J, Chapman H. 2008. A population estimate of the endangered chimpanzee *Pan troglodytes vellerosus* in a Nigerian montane forest: implications for conservation. *Oryx* 42(3):448-451.

Becquet C, Patterson N, Stone AC, Przeworski M, Reich D. 2007. Genetic structure of chimpanzee populations. *PLoS Genetics* 3(4):e66.

Bijlsma R, Bundgaard J, Boerema A. 2000. Does inbreeding affect the extinction risk of small populations?: predictions from Drosophila. *Journal of Evolutionary Biology* 13(3):502-514.

BirdLife International. 2013. *Important Bird Areas factsheet: Gashaka-Gumti National Park*. http://www.birdlife.org (accessed 06/06/2013)

Bland M. 2000. An introduction to medical statistics. Oxford University Press.

Boesch C. 1994. Cooperative hunting in wild chimpanzees. *Animal Behaviour* 48(3):653-667.

Boesch C, Boesch H. 1990. Tool use and tool making in wild chimpanzees. *Folia Primatologica* 54(1-2):86-99.

Boesch C, Marchesi P, Marchesi N, Fruth B, Joulian F. 1994. Is nut cracking in wild chimpanzees a cultural behaviour? *Journal of Human Evolution* 26(4):325-338.

Boesch C, Crockford C, Herbinger I, Wittig R, Moebius Y, Normand E. 2008. Intergroup conflicts among chimpanzees in Tai National Park: lethal violence and the female perspective. *American Journal of Primatology* 70(6):519-532.

Bradley BJ, Chambers KE, Vigilant L. 2001. Accurate DNA-based sex identification of apes using non-invasive samples. *Conservation Genetics* 2(2):179-181.

Bradley BJ, Doran-Sheehy DM, Vigilant L. 2007. Potential for female kin associations in wild western gorillas despite female dispersal. *Proceedings of the Royal Society B: Biological Sciences* 274(1622):2179-2185.

Britt A, Welch C, Katz A. 2004. Can small, isolated primate populations be effectively reinforced through the release of individuals from a captive population? *Biological Conservation* 115(2):319-327.

Broquet T, Petit E. 2004. Quantifying genotyping errors in noninvasive population genetics. *Molecular Ecology* 13(11):3601-3608.

Brouat C, Sennedot F, Audiot P, Leblois R, Rasplus JY. 2003. Fine-scale genetic structure of two carabid species with contrasted levels of habitat specialization. *Molecular Ecology* 12(7):1731-1745.

Campbell G, Kuehl H, N'Goran Kouamé P, Boesch C. 2008. Alarming decline of West African chimpanzees in Côte d'Ivoire. *Current Biology* 18(19):R903-R904.

Caro TM, Laurenson MK. 1994. Ecological and Genetic Factors in Conservation: A Cautionary Tale. *Science* 263(5146):485-486.

Caughley G. 1994. Directions in conservation biology. *Journal of Animal Ecology* 63:215-244.

Chapman JD, Chapman H. 2001. The Forest Flora of Taraba and Adamawa States, Nigeria: An Ecological Account and Plant Species Checklist: Department of Plant and Microbial Sciences, University of Canterbury, Christchurch, New Zealand.

Charlesworth B, Charlesworth D. 1999. The genetic basis of inbreeding depression. *Genetical Research* 74(3):329-340.

Charpentier MJ, Widdig A, Alberts SC. 2007. Inbreeding depression in non-human primates: a historical review of methods used and empirical data. *American Journal of Primatology* 69(12):1370-1386.

Chen X. 1993. Comparison of inbreeding and outbreeding in hermaphroditic Arianta arbustorum (L.)(land snail). *Heredity* 71(5):456-461.

Clarke AL, Sæther B-E, Røskaft E. 1997. Sex Biases in Avian Dispersal: A Reappraisal. *Oikos* 79(3):429-438.

Clark CJ, Poulsen JR, Malonga R, Elkan JPW. 2009. Logging Concessions Can Extend the Conservation Estate for Central African Tropical Forests. *Conservation Biology* 23(5):1281-1293.

Clutton-Brock T. 1989. Female transfer and inbreeding avoidance in social mammals. *Nature* 337:70-72.

Coltman DW, Pilkington JG, Smith JA, Pemberton JM. 1999. Parasite-mediated selection against inbred Soay sheep in a free-living, island population. *Evolution* 53:1259-1267.

Conservation International 2013. *Guinean Forests of West Africa*. http://www.conservation.org/where/priority_areas/hotspots/africa/Guinean-Forests-of-West-Africa/Pages/default.aspx (accessed 06/06/2013).

Cornuet J-M, Piry S, Luikart G, Estoup A, Solignac M. 1999. New methods employing multilocus genotypes to select or exclude populations as origins of individuals. *Genetics* 153(4):1989-2000.

Coulon A, Cosson J-F, Morellet N, Angibault J-M, Cargnelutti B, Galan M, Aulagnier S, Hewison A. 2006. Dispersal is not female biased in a resource-defence mating ungulate, the European roe deer. *Proceedings of the Royal Society B: Biological Sciences* 273(1584):341-348.

Couvet D. 2002. Deleterious effects of restricted gene flow in fragmented populations. *Conservation biology* 16(2):369-376.

Crnokrak P, Roff DA. 1999. Inbreeding depression in the wild. *Heredity* 83(3):260-270.

Davies KF, Margules CR, Lawrence JF. 2000. Which traits of species predict population declines in experimental forest fragments? *Ecology* 81(5):1450-1461.

Dewoody J, Nason JD, Hipkins VD. 2006. Mitigating scoring errors in microsatellite data from wild populations. *Molecular Ecology Notes* 6(4):951-957.

Dixo M, Metzger JP, Morgante JS, Zamudio KR. 2009. Habitat fragmentation reduces genetic diversity and connectivity among toad populations in the Brazilian Atlantic Coastal Forest. *Biological Conservation* 142(8):1560-1569.

Dixon JD, Oli MK, Wooten MC, Eason TH, McCown JW, Cunningham MW. 2007. Genetic consequences of habitat fragmentation and loss: the case of the Florida black bear (*Ursus americanus floridanus*). *Conservation Genetics* 8(2):455-464.

Dobson FS. 1982. Competition for mates and predominant juvenile male dispersal in mammals. *Animal Behaviour* 30(4):1183-1192.

Douadi MI, Gatti S, Levrero F, Duhamel G, Bermejo M, Vallet D, Menard N, Petit EJ. 2007. Sex-biased dispersal in western lowland gorillas (*Gorilla gorilla gorilla*). *Molecular Ecology* 16(11):2247-2259.

Efron B. 1983. Estimating the error rate of a prediction rule: improvement on cross-validation. *Journal of the American Statistical Association* 78(382):316-331.

Ely JJ, Frels WI, Howell S, Izard MK, Keeling ME, Lee DR. 2006. Twinning and heteropaternity in chimpanzees (*Pan troglodytes*). *American Journal of Physical Anthropology* 130(1):96-102.

Emery Thompson M, Jones JH, Pusey AE, Brewer-Marsden S, Goodall J, Marsden D, Matsuzawa T, Nishida T, Reynolds V, Sugiyama Y and others. 2007. Aging and Fertility Patterns in Wild Chimpanzees Provide Insights into the Evolution of Menopause. *Current biology : CB* 17(24):2150-2156.

Epps CW, Palsbøll PJ, Wehausen JD, Roderick GK, Ramey RR, McCullough DR. 2005. Highways block gene flow and cause a rapid decline in genetic diversity of desert bighorn sheep. *Ecology Letters* 8(10):1029-1038.

Eriksson J, Hohmann G, Boesch C, Vigilant L. 2004. Rivers influence the population genetic structure of bonobos (*Pan paniscus*). *Molecular Ecology* 13(11):3425-3435.

Eriksson J, Siedel H, Lukas D, Kayser M, Erler A, Hashimoto C, Hohmann G, Boesch C, Vigilant L. 2006. Y-chromosome analysis confirms highly sex-biased dispersal and suggests a low male effective population size in bonobos (*Pan paniscus*). *Molecular Ecology* 15(4):939-949.

Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 14(8):2611-2620.

Excoffier L, Lischer HE. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* 10(3):564-567.

.

Fahrig L. 2002. Effect of habitat fragmentation on the extinction threshold: a synthesis. *Ecological Applications* 12(2):346-353.

Falconer D, Mackay T. 1996. Introduction to quantitative genetics. Essex, England: Longman.

Favre L, Balloux F, Goudet J, Perrin N. 1997. Female-biased dispersal in the monogamous mammal *Crocidura russula*: evidence from field data and microsatellite patterns. *Proceedings of the Royal Society of London Series B: Biological Sciences* 264(1378):127-132.

Ferber D. 2000. Human Diseases Threaten Great Apes. *Science* 289(5483):1277-1278.

Fischer A, Pollack J, Thalmann O, Nickel B, Pääbo S. 2006. Demographic history and genetic differentiation in apes. *Current Biology* 16(11):1133-1138.

Fontanillas P, Petit E, Perrin N. 2004. Estimating sex-specific dispersal rates with autosomal markers in hierarchically structured populations. *Evolution* 58(4):886-894.

Fowler A, Sommer V. 2007. Subsistence technology of Nigerian chimpanzees. *International Journal of Primatology* 28(5):997-1023.

Fowler A, Koutsioni Y, Sommer V. 2007. Leaf-swallowing in Nigerian chimpanzees: evidence for assumed self-medication. *Primates* 48(1):73-76.

Fowler A, Pascual-Garrido A, Buba U, Tranquilli S, Akosim C, Schöning C, Sommer V. 2011. Panthropology of the Fourth Chimpanzee: A Contribution to Cultural Primatology. In: Sommer V, Ross C, editors. *Primates of Gashaka*: Springer. pp 451-492.

Frankel OH, Soulé ME. 1981. Conservation and evolution: Cambridge University Press.

Frankham R. 1995. Effective population size/adult population size ratios in wildlife: a review. *Genetical Research* 66(02):95-107.

Frankham R. 1996. Relationship of genetic variation to population size in wildlife. *Conservation Biology* 10(6):1500-1508.

Frankham R. 2005. Genetics and extinction. *Biological Conservation* 126(2):131-140.

Frankham R, Briscoe DA, Ballou JD. 2002. Introduction to conservation genetics: Cambridge University Press.

Franklin IR, Frankham R. 1998. How large must populations be to retain evolutionary potential? *Animal Conservation* 1(1):69-70.

Gagneux P, Boesch C, Woodruff D. 1997. Microsatellite scoring errors associated with noninvasive genotyping based on nuclear DNA amplified from shed hair. *Molecular Ecology* 6(9):861-868.

Gandon S, Michalakis Y. 2001. Multiple causes of the evolution of dispersal. In: Clobert J, Danchin E, Dhondt A, Nichols J, editors. *Dispersal*. Oxford University Press.

Gauffre B, Petit E, Brodier S, Bretagnolle V, Cosson JF. 2009. Sex-biased dispersal patterns depend on the spatial scale in a social rodent. *Proceedings of the Royal Society B: Biological Sciences* 276(1672):3487-3494.

Gerlach G, Musolf K. 2000. Fragmentation of Landscape as a Cause for Genetic Subdivision in Bank Voles. *Conservation biology* 14(4):1066-1074.

Ghobrial L, Lankester F, Kiyang JA, Akih AE, de Vries S, Fotso R, Gadsby EL, Jenkins PD, Gonder MK. 2010. Tracing the origins of rescued chimpanzees reveals widespread chimpanzee hunting in Cameroon. *BMC Ecology* 10(1):2.

Gilpin ME, Soulé ME. 1986. Minimum viable populations: processes of species extinction. In: Soule ME, editor. Conservation biology: the science of scarcity and diversity: Sinauer Associates. pp 19-34.

Goldberg TL, Wrangham RW. 1997. Genetic correlates of social behaviour in wild chimpanzees: evidence from mitochondrial DNA. *Animal Behaviour* 54(3):559-570.

Gonder M, Disotell T. 2006. Contrasting phylogeographic histories of chimpanzees in Nigeria and Cameroon: a multilocus analysis. In: Lehman S, Fleagle J, editors. *Primate Biogeography*. Plenum/Kluwer Press. pp 129-161.

Gonder K, Oates J, Disotell T, Forstner M, Morales JC, Melnick DJ. 1997. A new west African chimpanzee subspecies? *Nature* 388:337.

Gonder MK, Disotell TR, Oates JF. 2006. New genetic evidence on the evolution of chimpanzee populations and implications for taxonomy. *International Journal of Primatology* 27(4):1103-1127.

Gonder MK, Locatelli S, Ghobrial L, Mitchell MW, Kujawski JT, Lankester FJ, Stewart C-B, Tishkoff SA. 2011. Evidence from Cameroon reveals differences in the genetic structure and histories of chimpanzee populations. *Proceedings of the National Academy of Sciences* 108(12):4766-4771.

Goodall J. 1986. The chimpanzees of Gombe: patterns of behavior: Belknap Press of Harvard University Press.

Goossens B, Funk SM, Vidal C, Latour S, Jamart A, Ancrenaz M, Wickings EJ, Tutin CE, Bruford MW. 2002. Measuring genetic diversity in translocation programmes: principles and application to a chimpanzee release project. *Animal Conservation* 5(3):225-236.

Goossens B, Setchell JM, Vidal C, Dilambaka E, Jamart A. 2003. Successful reproduction in wild-released orphan chimpanzees (*Pan troglodytes troglodytes*). *Primates* 44(1):67-69.

Goossens B, Chikhi L, Jalil M, Ancrenaz M, Lackman-Ancrenaz I, Mohamed M, Andau P, Bruford MW. 2005a. Patterns of genetic diversity and migration in increasingly fragmented and declining orang-utan (*Pongo pygmaeus*) populations from Sabah, Malaysia. *Molecular Ecology* 14(2):441-456.

Goossens B, Setchell J, Tchidongo E, Dilambaka E, Vidal C, Ancrenaz M, Jamart A. 2005b. Survival, interactions with conspecifics and reproduction in 37 chimpanzees released into the wild. *Biological Conservation* 123(4):461-475.

Goossens B, Setchell J, James S, Funk S, Chikhi L, Abulani A, Ancrenaz M, Lackman-Ancrenaz I, Bruford M. 2006. Philopatry and reproductive success in Bornean orang-utans (*Pongo pygmaeus*). *Molecular Ecology* 15(9):2577-2588.

Goudet J, Perrin N, Waser P. 2002. Tests for sex-biased dispersal using bi-parentally inherited genetic markers. *Molecular Ecology* 11(6):1103-1114.

Greengrass EJ. 2009. Chimpanzees are Close to Extinction in Southwest Nigeria. *Primate Conservation* 24(1):77-83.

Greenwood PJ. 1980. Mating systems, philopatry and dispersal in birds and mammals. *Animal Behaviour* 28(4):1140-1162.

de Groot NG, Otting N, Doxiadis GGM, Balla-Jhagjhoorsingh SS, Heeney JL, van Rood JJ, Gagneux P, Bontrop RE. 2002. Evidence for an ancient selective sweep in the MHC class I gene repertoire of chimpanzees. *Proceedings of the National Academy of Sciences* 99(18):11748-11753.

Groves CP. 1986. Systematics of the great apes. In: Swindler D, Erwin J, editors. *Comparative primate biology volume 1: systematics, evolution and anatomy*. New York: Alan R. Liss. pp 187-217.

Groves C. 2001. Primate taxonomy: Smithsonian, Washington.

Groves, C. P. 2005. Geographic variation within eastern chimpanzees (*Pan troglodytes c. schweinfrthii* Giglioli, 1872). *Australasian. Primatology*. 17:19–46.

Guillot G, Rousset F. 2013. Dismantling the Mantel tests. *Methods in Ecology and Evolution* 4(4):336-344.

Hamilton M. 2011. Population genetics: Wiley-Blackwell.

Hamilton W, May RM. 1977. Dispersal in stable habitats. *Nature* 269(5629):578-581.

Hanski I. 1998. Metapopulation dynamics. *Nature* 396(6706):41-49.

Hedrick PW. 2002. Lethals in Finite Populations. *Evolution* 56(3):654-657.

Hedrick PW, Fredrickson R. 2010. Genetic rescue guidelines with examples from Mexican wolves and Florida panthers. *Conservation Genetics* 11(2):615-626.

Henle K, Davies K, Kleyer M, Margules C, Settele J. 2004. Predictors of Species Sensitivity to Fragmentation. *Biodiversity & Conservation* 13(1):207-251.

Herbinger I, Boesch C, Rothe H. 2001. Territory characteristics among three neighboring chimpanzee communities in the Taï National Park, Côte d'Ivoire. *International Journal of Primatology* 22(2):143-167.

Hill K, Boesch C, Goodall J, Pusey A, Williams J, Wrangham R. 2001. Mortality rates among wild chimpanzees. *Journal of Human Evolution* 40(5):437-450.

Hubisz MJ, Falush D, Stephens M, Pritchard JK. 2009. Inferring weak population structure with the assistance of sample group information. *Molecular ecology resources* 9(5):1322-1332.

Hughes N, Rosen N, Gretsky N, Sommer V. 2011. Will the Nigeria-Cameroon chimpanzee go extinct? Models derived from intake rates of ape sanctuaries. In: Sommer V, Ross C, editors. *Primates of Gashaka*: Springer. pp 493-524.

Inskipp, T. 2005. Chimpanzee (*Pan troglodytes*). In: Caldecott J, Miles L, editors. *World Atlas of Great Apes and Their Conservation*. University of California Press. pp 53-82

IUCN 2013. *The IUCN Red List of Threatened Species. Version 2013.1*. http://www.iucnredlist.org (accessed 10/09/2013).

IUCN/SSC 2013. Guidelines for Reintroductions and Other ConservationTranslocations. Version 1.0. Gland, Switzerland: IUCN Species Survival Commission.

Jack KM, Fedigan L. 2004a. Male dispersal patterns in white-faced capuchins, *Cebus capucinus*: Part 1: patterns and causes of natal emigration. *Animal Behaviour* 67(4):761-769.

Jack KM, Fedigan L. 2004b. Male dispersal patterns in white-faced capuchins, *Cebus capucinus*: Part 2: patterns and causes of secondary dispersal. *Animal Behaviour* 67(4):771-782.

Jamieson IG, Allendorf FW. 2012. How does the 50/500 rule apply to MVPs? *Trends in Ecology & Evolution* 27(10):578-584.

Johansson M, Primmer CR, Sahlsten J, Merilä J. 2005. The influence of landscape structure on occurrence, abundance and genetic diversity of the common frog, *Rana temporaria*. *Global Change Biology* 11(10):1664-1679.

Keller LF, Waller DM. 2002. Inbreeding effects in wild populations. *Trends in Ecology & Evolution* 17(5):230-241.

Keller LF, Arcese P, Smith JN, Hochachka WM, Stearns SC. 1994. Selection against inbred song sparrows during a natural population bottleneck. *Nature* 372(6504):356-357.

Keyghobadi N. 2007. The genetic implications of habitat fragmentation for animals. *Canadian Journal of Zoology* 85(10):1049-1064.

Knight M, Oppen Mv, Smith H, Rico C, Hewitt G, Turner G. 1999. Evidence for male-biased dispersal in Lake Malawi cichlids from microsatellites. *Molecular Ecology* 8(9):1521-1527.

Köndgen S, Kühl H, N'Goran PK, Walsh PD, Schenk S, Ernst N, Biek R, Formenty P, Mätz-Rensing K, Schweiger B and others. 2008. Pandemic Human Viruses Cause Decline of Endangered Great Apes. *Current Biology* 18(4):260-264.

Kormos, R. 2003. West African chimpanzees: status survey and conservation action plan, Vol. 60. IUCN.

Kühl, H., F. Maisels, M. Ancrenaz, and E. Williamson. 2008. Best Practice Guidelines for Surveys and Monitoring of Great Ape Populations. IUCN, Gland, Switzerland in collaboration with the Center for Applied Biodiversity Science at Conservation International.

Lacy, R., M. Borbat, and J. Pollak. 1995. VORTEX: a stochastic simulation of the extinction process. Computer software, version 9.

Lambin X, Aars J, Piertney SB. 2001. Dispersal, intraspecific competition, kin competition and kin facilitation: a review of the empirical evidence. In: Clobert J, Danchin E, Dhondt A, JD N, editors. *Dispersal*: Oxford University Press, Oxford. p 110-122.

Lande R. 1988. Genetics and demography in biological conservation. *Science* 241(4872):1455-1460.

Lande R. 1993. Risks of Population Extinction from Demographic and Environmental Stochasticity and Random Catastrophes. *The American Naturalist* 142(6):911-927.

Lande R. 1998a. Demographic Stochasticity and Allee Effect on a Scale with Isotropic Noise. *Oikos* 83(2):353-358.

Lande R. 1998. Anthropogenic, ecological and genetic factors in extinction and conservation. *Researches on Population Ecology* 40(3):259-269.

Langergraber KE, Siedel H, Mitani JC, Wrangham RW, Reynolds V, Hunt K, Vigilant L. 2007. The genetic signature of sex-biased migration in patrilocal chimpanzees and humans. *PLoS One* 2(10):e973.

Langergraber KE, Prüfer K, Rowney C, Boesch C, Crockford C, Fawcett K, Inoue E, Inoue-Muruyama M, Mitani JC, Muller MN and others. 2012. Generation times in wild chimpanzees and gorillas suggest earlier divergence times in great ape and human evolution. *Proceedings of the National Academy of Sciences* 109(39):15716-15721.

Langergraber KE, Mitani J, Watts D, Vigilant L. 2013. Male–female socio-spatial relationships and reproduction in wild chimpanzees. *Behavioral Ecology and Sociobiology* 67(6):861-873.

Laporte NT, Stabach JA, Grosch R, Lin TS, Goetz SJ. 2007. Expansion of Industrial Logging in Central Africa. *Science* 316(5830):1451.

Lawson Handley L, Perrin N. 2007. Advances in our understanding of mammalian sex-biased dispersal. *Molecular Ecology* 16(8):1559-1578.

Le Galliard J-F, Gundersen G, Andreassen H, Stenseth N. 2006. Natal dispersal, interactions among siblings and intrasexual competition. *Behavioral Ecology* 17(5):733-740.

Leendertz FH, Ellerbrok H, Boesch C, Couacy-Hymann E, Mätz-Rensing K, Hakenbeck R, Bergmann C, Abaza P, Junglen S, Moebius Y. 2004. Anthrax kills wild chimpanzees in a tropical rainforest. *Nature* 430(6998):451-452.

Legendre S, Clobert J, Møller AP, Sorci G. 1999. Demographic stochasticity and social mating system in the process of extinction of small populations: the case of passerines introduced to New Zealand. *The American Naturalist* 153(5):449-463.

Lehmann J, Boesch C. 2004. To fission or to fusion: effects of community size on wild chimpanzee (*Pan troglodytes verus*) social organisation. *Behavioral Ecology and Sociobiology* 56(3):207-216.

Lehmann L, Perrin N. 2003. Inbreeding avoidance through kin recognition: choosy females boost male dispersal. *The American Naturalist* 162(5):638-652.

Leroy EM, Rouquet P, Formenty P, Souquière S, Kilbourne A, Froment J-M, Bermejo M, Smit S, Karesh W, Swanepoel R and others. 2004. Multiple Ebola Virus Transmission Events and Rapid Decline of Central African Wildlife. *Science* 303(5656):387-390.

Liberg O, Andrén H, Pedersen H-C, Sand H, Sejberg D, Wabakken P, Åkesson M, Bensch S. 2005. Severe inbreeding depression in a wild wolf *Canis lupus* population. *Biology letters* 1(1):17-20.

Lowen C, Dunbar RIM. 1994. Territory size and defendability in primates. *Behavioral Ecology and Sociobiology* 35(5):347-354.

Matsumoto-Oda A. 1999. Female choice in the opportunistic mating of wild chimpanzees (*Pan troglodytes schweinfurthii*) at Mahale. *Behavioral Ecology and Sociobiology* 46(4):258-266.

Matthews A, Matthews A. 2004. Survey of gorillas (*Gorilla gorilla gorilla*) and chimpanzees (*Pan troglodytes troglodytes*) in Southwestern Cameroon. *Primates* 45(1):15-24.

McGrew WC. 1994. Tools compared: the material of culture. In: Wrangham R, McGrew WC, de Waal F, Heltne P, editors. *Chimpanzee Cultures*. Harvard University Press.

Melbourne BA, Hastings A. 2008. Extinction risk depends strongly on factors contributing to stochasticity. *Nature* 454(7200):100-103.

Miller CR, Joyce P, Waits LP. 2002. Assessing allelic dropout and genotype reliability using maximum likelihood. *Genetics* 160(1):357-366.

Miller CR, Joyce P, Waits LP. 2005. A new method for estimating the size of small populations from genetic mark–recapture data. *Molecular Ecology* 14(7):1991-2005.

Miller PS.1994. Is inbreeding depression more severe in a stressful environment? *Zoo Biology* 13(3):195-208.

Miller P, Lacy R. 1999. VORTEX: a stochastic simulation of the extinction process. *User's manual, version* 8.

Mitani JC. 2006. Demographic influences on the behavior of chimpanzees. *Primates* 47(1):6-13.

Mitani JC. 2009. Male chimpanzees form enduring and equitable social bonds. *Animal Behaviour* 77(3):633-640.

Mitani JC, Amsler SJ. 2003. Social and Spatial Aspects of Male Subgrouping in a Community of Wild Chimpanzees. *Behaviour* 140(7):869-884.

Mitani JC, Merriwether DA, Zhang C. 2000. Male affiliation, cooperation and kinship in wild chimpanzees. *Animal Behaviour* 59(4):885-893.

Mitani JC, Watts DP, Muller MN. 2002. Recent developments in the study of wild chimpanzee behavior. *Evolutionary Anthropology: Issues, news, and reviews* 11(1):9-25.

Mitani JC, Watts DP, Amsler SJ. 2010. Lethal intergroup aggression leads to territorial expansion in wild chimpanzees. *Current biology* 20(12):R507-R508.

Morgan BJ, Abwe EE. 2006. Chimpanzees use stone hammers in Cameroon. *Current Biology* 16(16):R632-R633.

Morgan BJ, Adeleke A, Bassey T, Bergl R, Dunn A, Fotso R, Gadsby E, Gonder MK, Greengrass E, Koulagna DK. 2011. Regional action plan for the conservation of the Nigeria–Cameroon chimpanzee (*Pan troglodytes ellioti*). *IUCN/SSC Primate Specialist Group and Zoological Society of San Diego*.

Morgan D, Sanz C. 2007. Best practice guidelines for reducing the impact of commercial logging on great apes in Western Equatorial Africa: IUCN.

Morgan D, Sanz C, Greer D, Rayden T, Maisels F, Williamson EA. 2013. Great apes and FSC: Implementing 'ape friendly' practices in Central Africa's logging concessions. Gland, Switzerland: IUCN/SSC Primate Specialist Group.

Morin PA, Moore JJ, Chakraborty R, Jin L, Goodall J, Woodruff DS. 1994. Kin Selection, Social Structure, Gene Flow, and the Evolution of Chimpanzees. *Science* 265(5176):1193-1201.

Moritz C. 1999. Conservation units and translocations: strategies for conserving evolutionary processes. *Hereditas* 130(3):217-228.

Muller MN, Kahlenberg SM, Emery Thompson M, Wrangham RW. 2007. Male coercion and the costs of promiscuous mating for female chimpanzees. *Proceedings of the Royal Society B: Biological Sciences* 274(1612):1009-1014.

Nagy M, Heckel G, Voigt CC, Mayer F. 2007. Female-biased dispersal and patrilocal kin groups in a mammal with resource-defence polygyny. *Proceedings of the Royal Society B: Biological Sciences* 274(1628):3019-3025.

Narum SR. 2006. Beyond Bonferroni: less conservative analyses for conservation genetics. *Conservation Genetics* 7(5):783-787.

Nater A, Nietlisbach P, Arora N, van Schaik CP, van Noordwijk MA, Willems EP, Singleton I, Wich SA, Goossens B, Warren KS and others. 2011. Sex-Biased Dispersal and Volcanic Activities Shaped Phylogeographic Patterns of Extant Orangutans (genus: *Pongo*). *Molecular Biology and Evolution* 28(8):2275-2288.

Navidi W, Arnheim N, Waterman M. 1992. A multiple-tubes approach for accurate genotyping of very small DNA samples by using PCR: statistical considerations. *American Journal of Human Genetics* 50(2):347.

Newman D, Tallmon DA. 2001. Experimental evidence for beneficial fitness effects of gene flow in recently isolated populations. *Conservation biology* 15(4):1054-1063.

Nietlisbach P, Arora N, Nater A, Goossens B, Van Schaik CP, Krützen M. 2012. Heavily male-biased long-distance dispersal of orang-utans (genus: *Pongo*), as revealed by Y-chromosomal and mitochondrial genetic markers. *Molecular Ecology* 21(13):3173-3186.

Nishida T. 1968. The social group of wild chimpanzees in the Mahali Mountains. *Primates* 9(3):167-224.

Nishida T. 1990. The Chimpanzees of the Mahale mountains: sexual and life history strategies: University of Tokyo Press.

Nishimura A. 2003. Reproductive Parameters of Wild Female Lagothrix lagotricha. *International Journal of Primatology* 24(4):707-722.

Nunney L, Campbell KA. 1993. Assessing minimum viable population size: Demography meets population genetics. *Trends in Ecology & Evolution* 8(7):234-239.

Nyanganji G, Fowler A, McNamara A, Sommer V. 2011. Monkeys and Apes as Animals and Humans: Ethno-Primatology in Nigeria's Taraba Region. In: Sommer V, Ross C, editors. *Primates of Gashaka*: Springer. pp 101-134.

Oates J, Gadsby L, Jenkins P, Gonder K, Bocian C, Adeleke A. 2003. Nigeria. In: Kormos R, editor. *West African chimpanzees: status survey and conservation action plan*: IUCN. pp 123-132

Oates, J.F., Tutin, C.E.G., Humle, T., Wilson, M.L., Baillie, J.E.M., Balmforth, Z., Blom, A., Boesch, C., Cox, D., Davenport, T., Dunn, A., Dupain, J., Duvall, C., Ellis, C.M., Farmer, K.H., Gatti, S., Greengrass, E., Hart, J., Herbinger, I., Hicks, C., Hunt, K.D., Kamenya, S., Maisels, F., Mitani, J.C., Moore, J., Morgan, B.J., Morgan, D.B., Nakamura, M., Nixon, S., Plumptre, A.J., Reynolds, V., Stokes, E.J. & Walsh, P.D. 2008. *Pan troglodytes*. In: IUCN 2013. IUCN Red List of Threatened Species. Version 2013.1. www.iucnredlist.org (accessed 11/10/2013)

Oates J, Groves C, Jenkins P. 2009. The type locality of *Pan troglodytes vellerosus* (Gray, 1862), and implications for the nomenclature of West African chimpanzees. *Primates* 50(1):78-80.

O'Grady JJ, Brook BW, Reed DH, Ballou JD, Tonkyn DW, Frankham R. 2006. Realistic levels of inbreeding depression strongly affect extinction risk in wild populations. *Biological Conservation* 133(1):42-51.

Ogunjemite B, Ashimi T. 2010. Hunting and Trading in the Nigerian Chimpanzee (*Pan troglodytes vellerosus*) in Gashaka-Mambilla Region, Nigeria. *Ethiopian Journal of Environmental Studies and Management* 3(1).

van Oosterhout C, Hutchinson WF, Wills DP, Shipley P. 2004. Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* 4(3):535-538.

Packer C. 1979. Inter-troop transfer and inbreeding avoidance in Papio anubis. *Animal Behaviour* 27, Part 1(0):1-36.

Paetkau, D., W. Calvert, I. Stirling, and C. Strobeck. 1995. Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology* 4: 347-354.

Paetkau D, Slade R, Burden M, Estoup A. 2004. Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. *Molecular Ecology* 13(1):55-65.

Peakall R, Smouse PE. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6(1):288-295.

Perrin N, Goudet J. 2001. Inbreeding, kinship, and the evolution of natal dispersal. In: Clobert J, Danchin E, Dhondt A, Nichols J, editors. *Dispersal*. Oxford University Press, pp 123-142.

Piry S, Alapetite A, Cornuet J-M, Paetkau D, Baudouin L, Estoup A. 2004. GENECLASS2: a software for genetic assignment and first-generation migrant detection. *Journal of heredity* 95(6):536-539

Pompanon F, Bonin A, Bellemain E, Taberlet P. 2005. Genotyping errors: causes, consequences and solutions. *Nature Reviews Genetics* 6(11):847-846.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155(2):945-959.

Pusey AE, Wilson ML, Anthony Collins D. 2008. Human impacts, disease risk, and population dynamics in the chimpanzees of Gombe National Park, Tanzania. *American Journal of Primatology* 70(8):738-744.

Queller DC, Goodnight KF. 1989. Estimating relatedness using genetic markers. *Evolution*:258-275.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.http://www.R-project.org.

Ralls K, Ballou J. 1982. Effects of inbreeding on infant mortality in captive primates. *International Journal of Primatology* 3(4):491-505.

Ralls K, Ballou J. 1982. Effect of inbreeding on juvenile mortality in some small mammal species. *Laboratory Animals* 16(2):159-166.

Ralls K, Ballou JD, Templeton A. 1988. Estimates of lethal equivalents and the cost of inbreeding in mammals. *Conservation biology* 2(2):185-193.

Rathcke B, Real L. 1993. Autogamy and inbreeding depression in mountain laurel, *Kalmia latifolia* (*Ericaceae*). *American Journal of Botany*:143-146.

Redmond, I. 2001. Coltan boom, gorilla bust. The Impact of Coltan Mining on Gorillas and other Wildlife in Eastern DR Congo. Dian Fossey Gorilla Fund and Born Free Foundation. http://www.bornfree.org.uk/coltan (accessed 12/10/2013).

Reed DH. 2004. Extinction risk in fragmented habitats. *Animal Conservation* 7(02):181-191.

Reed DH. 2005. Relationship between population size and fitness. *Conservation biology* 19(2):563-568.

Reed DH, Frankham R. 2003. Correlation between fitness and genetic diversity. *Conservation biology* 17(1):230-237.

Reed DH, O'Grady JJ, Brook BW, Ballou JD, Frankham R. 2003. Estimates of minimum viable population sizes for vertebrates and factors influencing those estimates. *Biological Conservation* 113(1):23-34.

Saccheri I, Kuussaari M, Kankare M, Vikman P, Fortelius W, Hanski I. 1998. Inbreeding and extinction in a butterfly metapopulation. *Nature* 392(6675):491-494.

Shaffer ML. 1981. Minimum population sizes for species conservation. *BioScience*:131-134.

Shaffer M. 1987. Minimum viable populations: coping with uncertainty. In: Soule M, editor. *Viable populations for conservation*: Cambridge University Press. pp 69-86

Sharp PM, Shaw GM, Hahn BH. 2005. Simian immunodeficiency virus infection of chimpanzees. *Journal of Virology* 79(7):3891-3902.

Shimada MK, Hayakawa S, Fujita S, Sugiyama Y, Saitou N. 2008. Skewed matrilineal genetic composition in a small wild chimpanzee community. *Folia Primatologica* 80(1):19-32.

Shultz S, Opie C, Atkinson QD. 2011. Stepwise evolution of stable sociality in primates. *Nature* 479(7372):219-222.

Simberloff D. 1986. The Proximate Causes of Extinction. In: Raup DM, Jablonski D, editors. *Patterns and Processes in the History of Life*: Springer Berlin Heidelberg. pp 259-276.

Slate J, Kruuk L, Marshall T, Pemberton J, Clutton-Brock T. 2000. Inbreeding depression influences lifetime breeding success in a wild population of red deer (*Cervus elaphus*). *Proceedings of the Royal Society of London Series B: Biological Sciences* 267(1453):1657-1662.

Sommer V, Ross C. 2011. Exploring and Protecting West Africa's Primates: The Gashaka Gumti Primate Project in Context. In: Sommer V, Ross C, editors. *Primates of Gashaka*: Springer. pp 1-38.

Sommer V, Adanu J, Faucher I, Fowler A. 2004. Nigerian chimpanzees (*Pan troglodytes vellerosus*) at Gashaka: two years of habituation efforts. *Folia Primatologica* 75(5):295-316.

Spielman D, Brook BW, Frankham R. 2004. Most species are not driven to extinction before genetic factors impact them. *Proceedings of the National Academy of Sciences of the United States of America* 101(42):15261-15264.

Stacey PB, Taper M. 1992. Environmental variation and the persistence of small populations. *Ecological Applications*:18-29.

Starrfelt J, Kokko H. 2012. The thoery of dispersal under multiple influences. In: Clobert J, Baguette M, Benton T, Bullock J, editors. *Dispersal ecology and evolution*. Oxford: Oxford University Press.

Stephens PA, Sutherland WJ. 1999. Consequences of the Allee effect for behaviour, ecology and conservation. *Trends in Ecology & Evolution* 14(10):401-405.

Stumpf RM, Boesch C. 2005. Does promiscuous mating preclude female choice? Female sexual strategies in chimpanzees (*Pan troglodytes verus*) of the Taï National Park, Côte d'Ivoire. *Behavioral Ecology and Sociobiology* 57(5):511-524.

Sugiyama Y. 2004. Demographic parameters and life history of chimpanzees at Bossou, Guinea. *American Journal of Physical Anthropology* 124(2):154-165.

Sugiyama Y, Koman J. 1979. Social structure and dynamics of wild chimpanzees at Bossou, Guinea. *Primates* 20(3):323-339.

Sutton M, Anderson E. 2005. World atlas of great apes and their conservation. Cambridge University Press.

Taberlet P, Griffin S, Goossens B, Questiau S, Manceau V, Escaravage N, Waits LP, Bouvet J. 1996. Reliable genotyping of samples with very low DNA quantities using PCR. *Nucleic Acids Research* 24(16):3189-3194.

Taberlet P, Waits LP, Luikart G. 1999. Noninvasive genetic sampling: look before you leap. *Trends in Ecology & Evolution* 14(8):323-327.

Templeton AR, Shaw K, Routman E, Davis SK. 1990. The genetic consequences of habitat fragmentation. *Annals of the Missouri Botanical Garden*:13-27.

Thibault M, Blaney S. 2003. The Oil Industry as an Underlying Factor in the Bushmeat Crisis in Central Africa. *Conservation Biology* 17(6):1807-1813.

Thornhill NW. 1993. The natural history of inbreeding and outbreeding: theoretical and empirical perspectives. University of Chicago Press.

Traill LW, Bradshaw CJA, Brook BW. 2007. Minimum viable population size: A meta-analysis of 30 years of published estimates. *Biological Conservation* 139(1–2):159-166.

Tukur A, Adebayo AA, Galtima M. 2005. The land and people of the Mambilla Plateau. Nigeria: Heinemann Educational Books

Tutin CEG. 1979. Mating Patterns and Reproductive Strategies in a Community of Wild Chimpanzees (*Pan troglodytes schweinfurthii*). *Behavioral Ecology and Sociobiology* 6(1):29-38

Vrijenhoek RC. 1994. Genetic diversity and fitness in small populations. In: Loeschcke V, Jain SK, Tomiuk J, editors. *Conservation Genetics*: Birkhäuser Basel. pp 37-53.

de Waal F. 1984. Sex differences in the formation of coalitions among chimpanzees. *Ethology and Sociobiology* 5(4):239-255.

Waits LP, Luikart G, Taberlet P. 2001. Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. *Molecular Ecology* 10(1):249-256.

Wallis J. 1997. A survey of reproductive parameters in the free-ranging chimpanzees of Gombe National Park. *Journal of Reproduction and Fertility* 109(2):297-307.

Wallis J, Lee DR. 1999. Primate conservation: the prevention of disease transmission. *International Journal of Primatology* 20(6):803-826.

Walsh PD, Abernethy KA, Bermejo M, Beyers R, et al. 2003. Catastrophic ape decline in western equatorial Africa. *Nature* 422(6932):611-614.

Wattier R, Engel CR, Saumitou-Laprade P, Valero M. 1998. Short allele dominance as a source of heterozygote deficiency at microsatellite loci: experimental evidence at the dinucleotide locus Gv1CT in *Gracilaria gracilis* (Rhodophyta). *Molecular Ecology* 7(11):1569-1573.

Watts DP. 1998. Coalitionary Mate Guarding by Male Chimpanzees at Ngogo, Kibale National Park, Uganda. *Behavioral Ecology and Sociobiology* 44(1):43-55.

Watts DP, Mitani JC. 2001. Boundary patrols and intergroup encounters in wild chimpanzees. *Behaviour* 138(3):299-328.

White L, Tutin CE. 2001. Why chimpanzees and gorillas respond differently to logging: a cautionary tale from Gabon. *African Rain Forest Ecology and Conservation:*449-462.

Whiten A, Goodall J, McGrew WC, Nishida T, Reynolds V, Sugiyama Y, Tutin CEG, Wrangham RW, Boesch C. 2001. Charting Cultural Variation in Chimpanzees. *Behaviour* 138(11/12):1481-1516.

Wilkie D, Shaw E, Rotberg F, Morelli G, Auzel P. 2000. Roads, Development, and Conservation in the Congo Basin. *Conservation biology* 14(6):1614-1622.

Williams JM, Oehlert GW, Carlis JV, Pusey AE. 2004. Why do male chimpanzees defend a group range? *Animal Behaviour* 68(3):523-532.

Wilson ML, Wrangham RW. 2003. Intergroup relations in chimpanzees. *Annual Review of Anthropology*:363-392.

Wilson ML, Hauser MD, Wrangham RW. 2001. Does participation in intergroup conflict depend on numerical assessment, range location, or rank for wild chimpanzees? *Animal Behaviour* 61(6):1203-1216.

Woodford MH, Butynski TM, Karesh WB. 2002. Habituating the great apes: the disease risks. *Oryx* 36(02):153-160.

Wrangham R. 1994. Ecology, Diversity and Culture. In: Wrangham R, McGrew WC, de Waal F, Heltne P, editors. *Chimpanzee Cultures*. Harvard University Press.

Wroblewski EE, Murray CM, Keele BF, Schumacher-Stankey JC, Hahn BH, Pusey AE. 2009. Male dominance rank and reproductive success in chimpanzees, *Pan troglodytes schweinfurthii*. *Animal Behaviour* 77(4):873-885.

WWF 2013. *Great apes and logging report*. http://www.wwf.com (accessed 15/10/2013).

Zar JH. 1999. Biostatistical analysis, 4th edition: Pearson Education India.

Zhang B-W, Li M, Ma L-C, Wei F-W. 2006. A widely applicable protocol for DNA isolation from fecal samples. *Biochemical genetics* 44(11-12):494-503.

Appendix

Protocol for DNA extraction adapted from Zhang *et al.* (2006)

1. 1-1.5 g of faeces was weighed into a 15 ml tube.
2. 5 ml of tech grade ethanol was added and the sample was vortexed and centrifuged at 13,000 rpm for 2 min.
3. 5 ml of TE Buffer (10 mM Tris, 1 mM EDTA, pH 8) was added and the sample was vortexed and centrifuged at 13,000 rpm for 2 min.
4. 3 ml of TNE Buffer (10 m mol/L Tris-Cl, 0.5% SDS, 1 m mol/L CaCl2) and 50 µl of proteinase K (20 mg/mL) were added to the centrifuge tube and incubated for 2 hours.
5. The lysate was centrifuged at 13,000 rpm for 1 min.
6. The supernatant was added to a new 15 ml centrifuge tube containing 3 g of potato starch, then continuously vortexed for 1 min and incubated at room temperature for 1 min.
7. The tube was centrifuged at 13,000 rpm for 3 min.
8. 600 µl of supernatant was transferred to a new 2 ml tube and 150 µl of NaCl (3.5 mol/L) solution and 250 µl of CTAB (0.7 M NaCl, 10% cetyl trimethyl-ammonium bromide) solution were added.
9. The tube was incubated at 70°C for 10 min
10. 500 µl of the extract was added to 500 µl of phenol:chloroform:isoamyl alcohol (25:24:1) mixed by inverting and then centrifuged at 13,500 rpm to separate.
11. The supernatant was transferred to new 2 ml tube and step 10 was repeated.
12. An equal volume of binding buffer (4 M guanidine hydrochloride, 1 M potassium acetate, pH 5.5) was added to the tube, mixed gently, and applied to a spin column then loaded in a 2 mL microcentrifuge tube and centrifuged at 13,500 rpm for 30 secs.
13. The filter membrane was washed twice by centrifuging at 13,000 rpm for 1 min after applying 600 µl of 75% ethanol.
14. The DNA was eluted with 200 µL TE, and 50µg/mL RNase was added.

Qiagen protocol for extracting DNA

1. 180–220 mg of stool was weighed and placed in a 2 ml microcentrifuge tube and the tube was put on ice.
2. 1.6 ml of Buffer ASL was added to each stool sample and vortexed continuously for 1 min or until the stool sample was thoroughly homogenized.
3. Sample was centrifuged at 13,500 rpm for 1 min to pellet stool particles.
4. 1.4 ml of the supernatant was pipetted into a new 2 ml microcentrifuge tube and the pellet was discarded.
5. One InhibitEX Tablet was added to each sample and vortexed immediately and continuously for 1 min or until the tablet was completely suspended. Suspension was incubated for 1 min at room temperature.

6. Sample was centrifuged at 13,500 rpm for 3 min to pellet stool particles and inhibitors bound to InhibitEX matrix.
7. Immediately after the centrifuge stopped, all of the supernatant was pipetted into a new 1.5 ml microcentrifuge tube and the pellet was discarded. The sample was centrifuged at 13,500 rpm for 3 min.
8. 25 µl of proteinase K was pipetted into a new 2 ml microcentrifuge tube.
9. 600 µl of supernatant from step 7 was added to the 2 ml microcentrifuge tube containing proteinase K.
10. 600 µl of Buffer AL was added and vortexed for 15 s.
11. The solution was incubated at 70°C for 10 min.
12. 600 µl of ethanol (96–100%) was added to the lysate, and mixed by vortexing.
13. 600 µl of lysate from step 12 was added to a QIAamp spin column in a collection tube. The sample was centrifuged at 13,500 rpm for 1 min. The spin column was place in a new 2 ml collection tube and the previous tube was discarded.
14. A second aliquot of 600 µl of lysate was added to the spin column and centrifuged at 13,500 rpm for 1 min. The QIAamp spin column was placed in a new 2 ml collection tube, and the previous tube containing the filtrate was discarded.
15. Step 14 was repeated to load the third aliquot of the lysate onto the spin column.
16. 500 µl of Buffer AW1 was added to the spin column and centrifuged at 13,500 rpm for 1 min. The spin column was placed in a new collection tube and the previous one was discarded.
17. 500 µl of Buffer AW2 was added and centrifuged at 13,500 rpm for 3 min. The collection tube containing the filtrate was discarded.
18. The spin column was placed in a new 2 ml collection tube and centrifuged at 13,500 rpm for 1 min and the previous collection tube with the filtrate was discarded.
19. The QIAamp spin column was transferred into a labeled 1.5 ml microcentrifuge tube and 200 µl of Buffer AE was pipetted directly onto the QIAamp membrane. The tube was incubated for 1 min at room temperature, then centrifuged at 13,500 rpm for 1 min to elute DNA.